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A.M.A. JOURNAL OF

DISEASES OF CHILDREN

Society for Pediatric Research

Twenty-Eighth Annual Meeting
Atlantic City, N. J.
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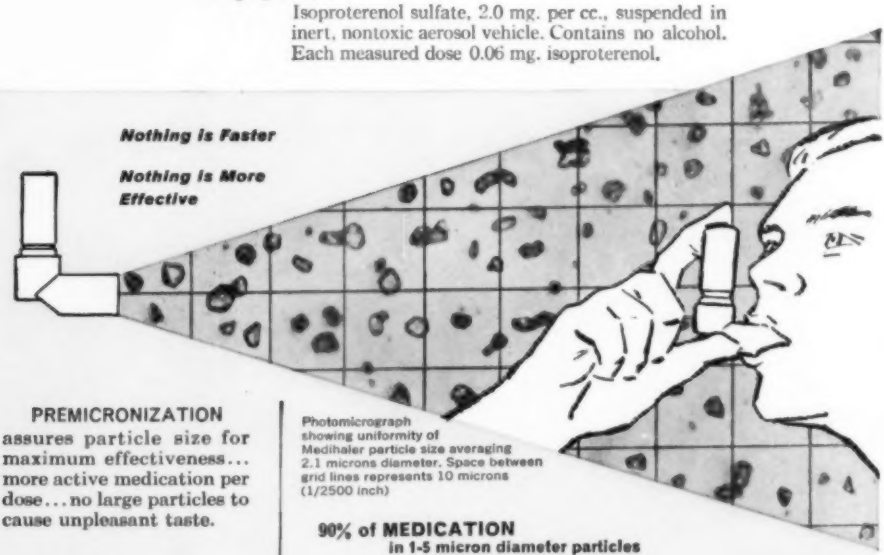

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A. M. A.
Journal of Diseases of Children

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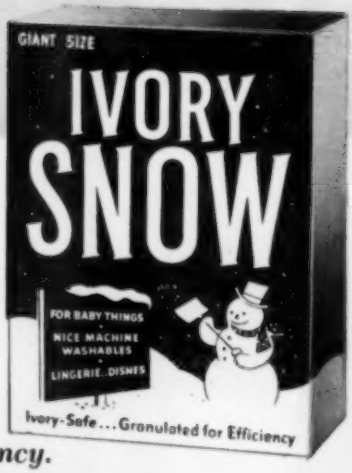

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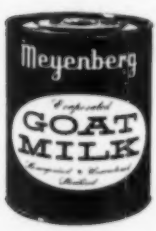

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Presidential Address, Society for Pediatric Research

EDWARD L. PRATT, M.D., Dallas, Texas

It is a great pleasure to me to have been chosen President of the Society for Pediatric Research, particularly because it grants me the opportunity to express publicly, no matter how inadequately, my gratitude to many people—to my parents, who presented the acquisition of knowledge as a desirable thing, to my wife, whose encouragement and other virtues should not be extolled here, and to my teachers.

Today I would like to discuss teaching and teachers.

What material can we teach? What can not or should not be taught? It would seem unnecessary to stress that one can truly teach only those things about which he has some knowledge.

Consider the clinical situation anuria. Here death is usually due to the disintegration of the structure of the internal environment. Therefore, the logical approach is to preserve the volume and the composition of the body fluids. The volume will be maintained when intake equals outgo, which in anuria is essentially the loss of insensible water from the lungs and skin. Thus the required information is the magnitude and the regulation of insensible water loss. Sanctorius, the father of instrumental medicine, spent nearly thirty years in his balance study of this function around 1600. Thus we see that the revolutionary penetration of accurate measurements into clinical medicine, decried by some even today, is not nearly so recent as we are often led to believe. Sanctorius stated: "The physician who considers only the sensible supplies and evacuations and knows nothing of the waste that is made daily by insensible perspiration will only deceive his patient and never cure him." In medicine, as elsewhere, some methods are "old but still good." The insensible water loss of infants, derived from

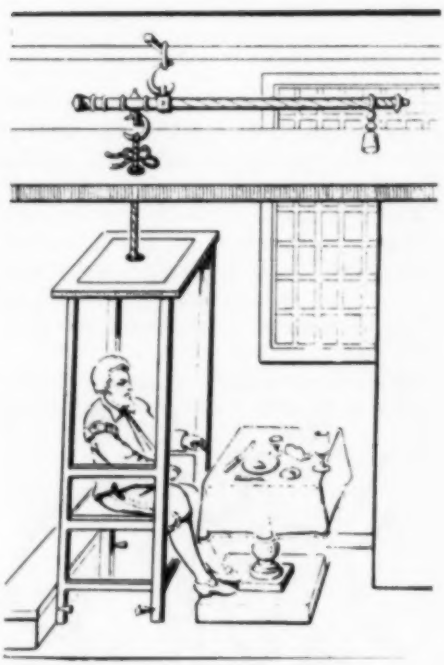

Fig. 1.—Sanctorius in his weighing chair.

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From the Department of Pediatrics, University of Texas Southwestern Medical School, The Children's Medical Center, and Parkland Memorial Hospital.

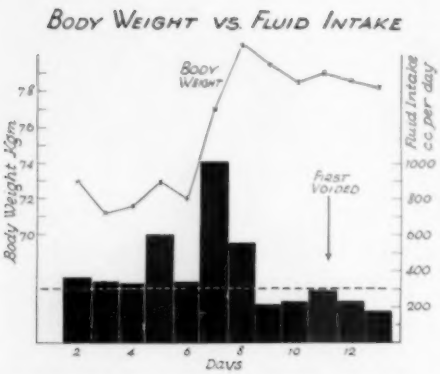

Fig. 2.—Body weight versus fluid intake for a child who was anuric for 11 days.

weight measurements, equals approximately 42 ml. per 100 Cal. expended. The application of such studies to patients with anuria is apparent from the data presented in Figure 2. Changes in body weight represent changes in body water, and the dashed line indicates the level of insensible water loss. As predicted, when water intake exceeds insensible water loss, body water increases; conversely, body water decreases when intake is less than insensible water loss. How clear it is that these simple investigations furnish the basis for understanding this important feature of body water expenditure. Here teaching is both simple and effective.

Contrast this situation with those clinical areas in which the fundamental underlying facts are unknown. These constitute the "inadequate information syndromes." The "inadequate information syndromes" have certain characteristics: 1. Frequently the symptoms are a selection from the conglomeration of vague complaints almost universally experienced by children. 2. The incidence of a given illness from these syndromes varies widely; a few doctors deny the existence of the disease under discussion, whereas others utilize it to cover many obscure conditions. 3. Widely differing treatments are reported to be successful, although not for long nor by many physicians. 4. Largely among these syndromes the belief prevails that experience with a large enough number of such patients will, in some nebulous way, allow the doctor to divine what to do without knowing why.[1] Yet, all too frequently, there is a plea for more teaching, more "education," in these unsatisfactory clinical situations. In these areas, where the basic facts are admittedly

Fig. 3*a*.—A principle of teaching (1)

Fig. 3*b*.—A principle of teaching (2)

unknown, more teaching is no solution, any more than more "education" in infectious diseases would have solved infectious diseases before the era beginning with Koch and Pasteur.

Thus it is evident that we can teach, in a real sense, only scientific medicine, which derives its being from research. With the crowded curriculum of today, care should be taken not to dissipate education into areas where real teaching is not possible. Equally important, time should not be wasted on trivia which any student can acquire for himself—a student need not be "taught" everything he ought to know.

Certainly I do not feel that I know all the principles of teaching, but from a careful review of my own experiences with some of what I believe to be the finest teachers in pediatrics—Gamble, Butler, Darrow, Holt, and many more—three features stand out. I remember their exposition of principles and their modes of thinking rather than the facts they imparted. All regarded "the mind of the student not as a bucket to be filled, but rather as a fire to be kindled." Each of these men was dissatisfied with anything short of a first rate performance. By their personal examples, by the atmosphere they created, and by my efforts to fit into their laboratories, I learned the Philosophy of the First Rate, a philosophy which furnishes a standard of values and judgments for all human activities. Education must, at the very least, lead our students to recognize, to desire and to pursue the First Rate.[2]

Fig. 4.—The problem of the 'nine dots.'

Observation of Dr. Powers and Dr. Crothers in action made it clear that, rather than any conflict existing between scientific medicine and compassion for patients, knowledge of disease enhances the empathy and psychotherapeutic effectiveness of the physician.

Dr. Crothers imparted a benign arrogance, a form of pride, that I consider essential. This is: "That while a specialist may be in a better position to offer detailed criticism of a procedure, the rest of us, provided we are truly interested in the well-being of children, are abundantly able to form sound counclusions as to the general validity of that procedure." [3]

Brilliant investigations and advances in medicine are not alone the result of persistence, equipment, and dollars, but depend in a real measure upon the point of view of the leader, as illustrated by the following story. During my freshman year at the Massachusetts Institute of Technology, our professor of mathematics began his course by giving the following assignment. "Connect these nine dots with four straight lines without lifting the pencil from the paper." By the next morning we were all in a fit of frustration. One scholar presented a highly technical treatise proving that a solution was impossible. This was not too unlikely at

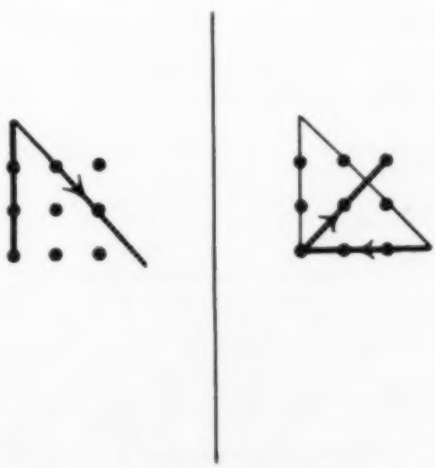

Fig. 5.—The solution of the 'nine dots' problem.

M. I. T. The professor then went to the blackboard and showed us how to solve the problem. One student protested, "That is not fair! You went outside the dots." The professor replied, "Who said you couldn't? Gentlemen, we are not here to go through old routines. Do not let your thinking be contained in a small square of knowledge. Learn to go 'outside the dots' and you may be the one to solve some of man's most baffling problems."

It may not be too difficult for you, as individuals, to make your investigations and teachings scientific and first rate. If you have any fruitful ideas about them, you may devote more attention to the "inadequate information syndromes."

But what about this Society? Its primary function is to stimulate new investigators who will 'go outside the dots'. Should not the Society concern itself with the attraction of our finest students into research? By analogy with pediatrics, must we not supervise the growth and development of these people during their phase of dependency?

Research provides the material for valid teaching, and from this Society come many of the teachers. Should we have no further concern with it? Is it wise for Societies such as this one to permit the major control of medical education to go, by default, to others more remote from teaching and less competent to judge it? All too frequently these other groups, with a different major interest, fail to distinguish between education and vocational training. Yet as President Dodds of Princeton observed, "Our vexed world will not be saved by the purely vocational mind."

Ladies and gentlemen, I believe we must assert our 'benign arrogance' and must concern ourselves with these vital affairs, if, as a Society, we wish to achieve our greatest usefulness to pediatrics.

5323 Hines Boulevard (19).

REFERENCES

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2. Livingstone, R.: What Is Education? in Proceedings of the First World Conference on Medical Education, New York, Oxford University Press, 1954, p. 12.

3. Crothers, B.: Disorders of the Nervous System in Clinical Pediatrics, New York, D. Appleton & Company, Vol. 4, 1926.

Transactions of the Society for Pediatric Research

Twenty-Eighth Annual Meeting

Atlantic City, N. J., May 6 and 7, 1958

SCIENTIFIC PROGRAM

Papers Read in Plenary Sessions

1. **Disturbance in Tryptophane Metabolism in Phenylketonuria**
   Heinz Berendes,* J. A. Anderson, M. R. Zeigler,* and Diane Ruttenberg *
2. **Experiences with a Live Attenuated Measles Virus**
   Samuel L. Katz,* Donald N. Medaris Jr.,* and John F. Enders *
3. **Tissue Oxygen Tension During Periods of Extracorporeal Circulation**
   J. A. Manning,* K. Woodward,* P. V. Gabel,* and S. I. Schwartz *
4. **Observations on the Metabolic Effects of Glucagon and Growth Hormone (Human and Beef) in an Infant with Idiopathic Hypoglycemia and Hyperinsulinism**
   John F. Crigler Jr., John A. Knapp,* and Jean Chagnon *
5. **Osmotic Characteristics of Renal Papillae and Urine in Relation to Counter-Current Loop Schemes of Concentration of Urine**
   J. W. Th. Appelboom * and William A. Brodsky
6. **On the Origin of Variants of Human Amniotic Cells in Tissue Culture**
   Juan C. Rivadeneira * and Frederick C. Robbins
7. **Preliminary Observations with a Procedure for Defining Human Factors of Importance in the Medical Management of Children with Long-Term Illnesses**
   Stuart Flerlage,* Marilyn Bailey,* and Nathan B. Talbot
8. **Studies on the Hereditary Enzymatic Defect in Certain Drug-Induced Hemolytic Anemias**
   Ruth T. Gross, Paul A. Marks,* Ruth E. Hurwitz,* Judith Beasley,* and Moyra Sui *
9. **Cyanosis Due to an Abnormal Hemoglobin**
   Park S. Gerald * and Louis K. Diamond
10. **The "Stuffy Nose Syndrome" of Premature Infants: An Example of Bacterial Viral Synergism**
    Heinz F. Eichenwald
11. **Mechanisms in Infantile Acrodynia (Pink Disease)**
    Donald B. Cheek
12. **Observations on the Control of Bilirubinemia**
    Stuart Danoff,* Audrey Boyer,* and L. Emmett Holt Jr.
13. **Congenital Malformations of the Heart in Siblings**
    Robert A. Miller * and Evangelia Zervopoulos *
14. **Morphological Changes in the Brain in Experimental Hypernatremia**
    Lawrence Finberg and Charles Luttrell *
15. **An Evaluation of Tm$_{c}$ H_2O in Children with Sickle-Cell Anemia**
    Charles F. Whitten * and Antoine A. Younes *
16. **Human Growth Hormone in Human Biologic Fluids**
    D. K. Keele,* H. Mansmann,* and R. Klein
17. **Factors Affecting Fluorescence of Antibody-Labelled Erythrocytes**
    JoAnne Whitaker,* Wolf W. Zuelzer, Abner F. Robinson,* and Margaret M. Evans *
18. **The Electronic Control of Extracorporeal Circuits by Muscle Action Potentials**
    Randolph Batson,* Sam E. Stephenson Jr.,* S. L. Clark,* and L. H. Montgomery *
19. **Coxsackie Infection in Infants and Children: 1. Clinical Aspects; 2. Pathology**
    D. M. Hosier * and W. A. Newton Jr.*
20. **Pulmonary Ventilation in Infants Less Than One Year of Age**
    T. K. Oliver Jr.,* R. S. Shaw,* and W. E. Wheeler
21. **Study of Juvenile Pernicious Anemia**
    Doris A. Howell *

* By Invitation

22. **Adenosine Triphosphatase Activity in the Cell Membranes of Normal and Nephrotic Rat Kidney Tubule Cells**
Herman W. Spater * and Vincent Drapiewski *

23. **The Distribution of Bromide in Body Tissues**
Stanley Hellerstein,* Carolyn J. Kaiser,* Daniel DeS. Darrow,* and Daniel C. Darrow

24. **A Human Pregnancy Plasma Adrenotropin: Its Identification as Chorionic Gonadotropin and Its Relation to Fetal Adrenal Enlargement**
Jonathan T. Lanman and Jenny Dinerstein *

25. **Platelet Factor 5: A Newly Identified Function of the Blood Platelets**
Irving Schulman, Carl H. Smith, Marion E. Erlandson,* and Jacqueline Wehman *

26. **Calcification Studies in Clinical Vitamin D Deficiency and in Hypophosphatemic Vitamin D-Refractory Rickets: The Induction of Calcium Deposition in Rachitic Cartilage Without the Administration of Vitamin D**
D. Fraser, D. W. Geiger,* J. D. Munn,* P. E. Slater,* R. Jahn,* and E. Liu *

27. **Studies in the Treatment of Acute Iron Poisoning**
Thomas R. C. Sisson and William R. Bronson *

28. **Estriol Excretion in Childhood: A Sensitive Index of Adrenocortical Function**
Walter R. Eberlein and Alfred M. Bongiovanni

29. **Principles Pertinent to Vaccination of Children Against Influenza**
A. V. Hennessy

30. **Urinary Excretion and Tissue Storage of Sulfated Mucopolysaccharides in Hurler's Syndrome**
Melvin M. Grumbach and Karl Meyer *

31. **An Unusual Case of Chronic Hypokalemia Associated with Renal Tubular Degeneration**
R. J. Slater, P. Azzopardi,* P. E. Slater,* and A. L. Chute

32. **A Simplified Test for Histoplasmosis Utilizing Precipitin-in-Gel Reactions**
Douglas C. Heiner *

33. **The Response of the Newborn to Major Surgery: Urinary Electrolyte, Nitrogen, and Water Losses**
Eleanor Colle MacLean * and Elsa Proehl Paulsen *

34. **"Vitamin D Activity" in Idiopathic Hypercalcemia**
Francis X. Fellers * and Robert Schwartz

35. **Methemoglobin Reduction in the Erythrocytes of Newborns: Further Evidence of "Enzymatic Immaturity" in the Newborn**
Jean P. Dawson * and Jane F. Desforges *

36. **Intracardiac Phonocardiography in the Diagnosis of Congenital Heart Disease**
David H. Lewis,* Ali Ertugrul,* George W. Deitz,* John D. Wallace,* James R. Brown Jr.,* and Abdol-Nabi Moghadam *

37. **Studies of Normal and Malignant Cells in Tissue Culture: The Effects of Heterologous Immune Serums upon HeLa Cells in Vitro and Rat-HeLa Tumors in Vivo**
Robert M. McAllister, Paul G. Grunmeier,* and Lewis L. Coriell

38. **Growth of Human Cancer Cells on in Vivo Tissue Culture**
Donald Pinkel * and G. David Baer *

39. **The Seromucoid Values in Hypothyroidism and Hyperthyroidism as Determined By a New Technique**
S. Harshman,* J. B. Sidbury Jr., and W. H. Price *

Papers Read in Special Sessions

40. **Pulmonary Valvular Stenosis with Intact Ventricular Septum in Infants**
Mary Jane Luke,* Catherine A. Neill,* and Helen B. Taussig

41. **The Effect of Penicillin on Acute Rheumatic Fever and Valvular Heart Disease**
Edward A. Mortimer Jr.,* Samuel Vaisman,* Augusto Schuster,* Julio Guasch,* Alberto Vignau,* Louis Rakita,* Charles H. Rammelkamp Jr.,* and Richard Krause

42. **Phonocardiographic Characteristics of Atrial Septal Defect**
Robert Eisenberg * and Herbert Hultgren *

43. **Basic Hemodynamics in Infants and Children**
Abraham M. Rudolph,* Milton H. Paul,* Glen C. Cayler,* and Julien I. E. Hoffman *

44. **Studies on the Type-Specific Immune Response to Group A Streptococcal Infections in Children**
Alan C. Siegel and Gene H. Stollerman

45. **Effect of Acetylcholine on Pulmonary Hypertension in Patients with Ventricular Septal Defects**

* By Invitation

I. Hunter Crittenden * and Forrest H. Adams

46. **The Evolution of Metabolic and Ventilatory Changes in Respiration in the First Two to Five Weeks of Life**
Mildred Stahlman

47. **Blood Oxygen Tension as a Measure of the Adequacy of Pulmonary Ventilation During the First Hour of Life**
Irwin Weisbrot *

48. **The Mechanism of Action of Phosphoglucomutase from Muscle and Yeast: Its Implication in Galactosemia**
Ernest E. McCoy * and Victor A. Najjar

49. **Observations on Glucuronic Acid Conjugation in Patients with Familial Nonhemolytic Jaundice and Their Families**
Barton Childs, James B. Sidbury Jr., and Claude Migeon

50. **Further Studies in Glucuronide Conjugation in the Newborn**
Audrey K. Brown,* Wolf W. Zuelzer, and Alfred Jay Bollet,* with the assistance of Herbert H. Burnett

51. **The Demonstration of Antibodies to Thyroglobulin (Autoimmunization Phenomenon) in Patients With Thyroid Disease**
Robert M. Blizzard and Warren E. Wheeler

52. **Giardiasis Causing the Celiac Syndrome: Clinical Observations and Studies of I^{131} Fat Absorption**
J. A. Cortner *

53. **A Familial Metabolic Disorder with an Unusual Polysaccharide Complex**
John M. Craig and L. Lahut Uzman *

54. **Studies of Familial Oxalosis**
T. H. Shepard,* E. G. Krebs,* and L. S. Lee *

55. **Defect of the Iodinating System in Congenital Goitrous Cretinism: Report of One Case with Biochemical Studies**
Heskel M. Haddad * and James B. Sidbury Jr.

56. **Study of the Effects of a Therapeutic Attempt at Complete Parenteral Alimentation in Infants**
LeRoy W. Matthews,* Wayne H. Borges,* Robert P. Bolande,* and Samuel Spector

57. **Nitrogen Utilization in Caloric- and Protein-Depleted Infants**
Mitchell I. Rubin, P. K. Mukherji,* and Philip L. Calcagno

58. **Growth and Body Composition of Piglets on Various Feedings**
L. J. Filer Jr., L. S. Baur,* and Helen Rezabek *

59. **The Febrile Seizure Threshold in Relation to Maturation of Brain Water, Electrolyte, and Acid-Base Balance of Newborn Animals**
J. Gordon Millichap

60. **Studies on the Mechanism of Production of Nephrosis by Aminonucleoside**
S. G. F. Wilson,* D. A. Goldthwait,* and Walter Heymann *

61. **Alterations in Bone Composition Following Nephrectomy in the Rat**
William H. Bergstrom and Eugene R. Jacobs *

62. **Total Body Water and Bromide Space Determinations in Premature Infants**
Ned W. Smull *

63. **Metabolic Alkalosis: A Specific Effect of Adrenocortical Hormones**
A. P. Grollman * and J. L. Gamble Jr.

64. **Meningitis in Newborns Caused by an Unclassified Gram-Negative Bacterium**
Jacob A. Brody,* Helen Moore,* and Elizabeth O. King *

65. **The Use of Latex Particles in the Serological Study of Bacterial Antigen-Antibody Systems**
Alfred L. Florman and Jeanne Scoma *

66. **Host-Graft Relationships Using S^{35} Methionine**
Frederick W. Barnes Jr., Milton T. Edgerton Jr.,* Harvey Kemp Jr.,* and William Seip *

67. **The Nature of "Immunological Paralysis" Induced by Pneumococcus Polysaccharides**
David Gitlin, Fernando Monckeberg,* and John M. Craig

68. **Scrofula Caused by Chromogenic Acid-Fast Mycobacteria**
John S. Chapman * and L. Ruth Guy *

69. **Isonicotinic Acid Hydrazide Levels in Children**
N. Matsaniotis,* C. Tsenghi,* and M. H. D. Smith

70. **A Preliminary Study of the Candida Albicans Hemagglutination Test and Its Potential Diagnostic Uses**
Nancy Huang *

71. **The Mechanism of Bacteriolysis by the Properdin System**
Ralph J. Wedgwood

* By Invitation

72. **Hemadsorption Viruses in Infections of the Respiratory Tract of Children**
Robert H. Parrott, Robert M. Chanock, Andrew Vargosko,* Alia Luckey,* Lotta Chi,* M. Katherine Cook,* Walter James,* Christine Cumming,* William O'Reilly,* William Stohlman,* and Gloria Eng *

73. **Serologic Response of Children to Asian Influenza Vaccine**
M. Michael Sigel,* Gloria G. Schlaepfer,* Raphael B. Shouger,* and Mildred C. Launer *

74. **Severe Generalized Disease in the Newborn Due to Coxsackie Virus Group B**
Sidney Kibrick and Kurt Benirschke *

75. **Disease in Monkeys Inoculated with ECHO Virus**
John H. Arnold * and John F. Enders *

76. **Alteration of Antigenicity of Poliovirus**
Katherine Sprunt * and Winifred M. Redman *

77. **The Relationship of Identifiable Viruses and the Clinical Pictures of Poliomyelitis and Aseptic Meningitis**
W. F. Howatt,* D. G. Dickinson,* G. R. DeMuth,* D. E. Craig,* and G. C. Brown *

78. **A Clinical, Epidemiologic and Laboratory Study of Illness Due to ECHO 9 Virus in a Housing Development**
Martha L. Lepow * and David H. Carver

79. **Isolation of Virus of Measles from Three Fatal Cases of Giant-Cell Pneumonia**
Kevin MacCarthy,* Anna Mitus,* William Cheatham,* and Thomas Peebles *

80. **Glutathione-Sensitive Chronic Nonspherocytic Hemolytic Anemia**
W. A. Newton Jr.* and J. C. Bass *

81. **Observations on the Glutathione (GSH) Stability of Red Blood Cells**
J. J. McGovern,* K. Isselbacher,* P. J. Rose,* and M. S. Grossman *

82. **The Antibody Elution Test in Heterospecific Pregnancies**
Victor C. Vaughan, Arthur E. McElfresh,* and Muzaffer Kurkcuoglu *

83. **Sequestration of Iron in the Lungs in Idiopathic Pulmonary Hemosiderosis**
Denman Hammond * and Jackson Crane *

84. **Alteration in the Electrophoretic Characteristics of Hemoglobin in "Paint Eaters"**
A. H. Tuttle and Charles Fitch *

85. **Thalassemia-like Syndromes Due to Recently Discovered Abnormal Hemoglobins**
Park S. Gerald * and Louis K. Diamond

86. **Studies in Sickle-Cell Anemia: Further Studies on Hepatic Function**
Angella D. Ferguson * and Roland B. Scott

87. **Studies of Kernicterus in Genetically Jaundiced Rats**
Lois Johnson,* Felicitas Sarmiento,* and Richard Day

Papers Read by Title

88. **Cause of Anemia in Erythroblastosis Fetalis**
Fred H. Allen Jr., Louis K. Diamond, and John M. Craig

89. **The Effects of Vascular Distention on Inflation of the Newborn Lung**
M. E. Avery,* N. R. Frank,* and I. Gribetz *

90. **The Use of a Magnetic Tape Recorder in Electromyography**
Henry W. Baird III

91. **Studies of Adenovirus Infections in Infants and Children**
Marc Beem,* J. T. Grayston,* and F. H. Wright

92. **Glycogen Synthesis and the Mechanism of Action of Insulin**
S. P. Bessman and J. Fitzgerald *

93. **Avitaminosis E in Cystic Fibrosis of the Pancreas: A Morphologic Study of Gastrointestinal and Striated Muscle**
W. A. Blanc, J. D. Reid,* and D. H. Andersen

94. **The Possible Mechanism and Site of Bilirubin Inhibition of Electron Transport**
Willet R. Bowen * and William J. Waters

95. **Acute Erythroblastopenia Due to Chloramphenicol**
Robert A. Bridges,* Hugh Dillon,* and Robert A. Good

96. **Limitations of the Postulate of Block of Hydrogen Ion Secretion in Explaining the Renal Action of Acetazoleamide**
W. A. Brodsky and R. Satran *

97. **Magnesium, Sodium, and Potassium in the Serum of Mothers and Their Newborns**
David F. Brown,* Edward Gillie,* Robert B. McGandy,* and Joseph T. Doyle *

98. **Observations on Some Serum Components in Mothers and Their Newborn Infants**
David F. Brown,* Edward Gillie,* Robert B. McGandy,* and Joseph T. Doyle *

99. **Immunological Study of Children Inoculated with Monovalent Asian Strain Influenza Vaccine**
Henry B. Bruyn and E. H. Lennette *

*By invitation

100. **BCG Vaccination in Newborns**
Ethel Cermak *

101. **Origin of Blood Ammonia in the Newborn**
R. L. Clemmens * and S. P. Bessman

102. **Urinary Steroid Excretion Patterns in the Neonatal Period**
Eleanor Colle * and Robert A. Ulstrom

103. **Studies of Normal and Malignant Cells in Tissue Culture: Alterations in Primate Nonprimate Cells Maintained in Tissue Culture as Measured by Antigenicity, Chromosome Number, and Tumor Production in Rats Pretreated with X-Ray and Cortisone**
Lewis L. Coriell, Robert M. McAllister, and Milton G. Tall *

104. **Glycogen Synthesis by Human Erythrocytes**
M. Cornblath, E. Y. Levin,* J. B. Sidbury Jr., and E. Marquetti *

105. **The Effect of Body Position on Respiratory Rate, Retraction Score, and Postnatal Weight Loss of Premature Infants**
J. A. Cortner,* J. C. Dower,* and W. A. Silverman

106. **Isolation of a Cytopathogenic Agent Resembling CA Virus from an Infant with Croup**
Henry G. Cramblett *

107. **The Transfer of Estrogens and Carbohydrates Across Guinea Pig Placenta Using an in Vivo Perfusion Technique**
Joseph Dancis, Mortimer Levitz,* and William L. Money *

108. **Psychiatric Studies of Children with Epilepsy**
Chester C. D'Autremont,* Ethel Walsh,* and Geraldine Rickard

109. **The Pattern of Fecal Fat Excretion in Premature Infants**
Murray Davidson * and Charles H. Bauer *

110. **The Occurrence of Asian Influenza in a Group of Cleveland Families**
Floyd W. Denny Jr., William S. Jordan Jr.,* Constance Curtiss,* Robert Oseasohn,* George F. Badger,* and John H. Dingle *

111. **Prolonged Pulsation of the Umbilical Cord After Birth, a Presumptive Sign of Neonatal Distress**
M. M. Desmond,* A. L. Megarity,* J. L. Kay,* and C. A. Brown *

112. **Gonadal Dysgenesis in Infants and Children**
Angelo M. DiGeorge * and James B. Arey

113. **Hypoelectrolytemia in Cold Weather in Infants and Young Children with Cystic Fibrosis of the Pancreas**
Paul A. DiSant'Agnese

114. **Cord Vein Blood Oxygen Saturations and Intelligence Quotients**
Donal Dunphy,* Vivian Pessin,* and Loren MacKinney

115. **Heart Disease Associated with Hemosiderosis and Chronic Anemia**
Mary Allen Engle, Henry P. Goldberg,* Gertrude Stern,* Irving Schulman, and Carl Smith

116. **Congenital Toxoplasmosis: Follow-Up Study**
Harry A. Feldman and Louise T. Miller *

117. **Heat-Labile Anti-Toxoplasma Serum Factors in Fetal Circulations**
Harry A. Feldman and Louise T. Miller

118. **The Postoperative Renal Excretion of Water in Infants**
Daniel C. Fleisher,* Wallace W. McCrory, and Leonard Bachman *

119. **Reproducibility of Three-Day Metabolic Balance Studies with Infants**
Samuel J. Fomon, Lora N. Thomas,* Robert L. Jensen,* and Charles D. May

120. **11-β Hydroxylase Deficiency in Congenital Adrenal Hyperplasia in the Absence of Hypertension**
Hortense M. Gandy * and E. Henry Keutmann *

121. **Studies in Cystic Fibrosis of the Pancreas: Amino Acids in Sweat**
Hossein K. Ghadimi * and Milton Stern *

122. **Quantitative Urine Bacteriology in Children**
G. D. Godenne,* C. B. Morrison,* and R. E. Cooke

123. **Placebo Versus Reserpine: Comparison of Value in Controlled Study of Maladjusted High-Grade Retarded Children**
Bruce D. Graham, Sidney Rosenblum,* Roger Callahan,* and Richard Deatrick *

124. **Studies on Glucuronide Conjugation and Diurnal Variation of Urinary 17-Hydroxycorticosteroids in the Neonatal Period**
Robert Greenberg,* Lytt I. Gardner, and Artelissa A. Lipfert *

125. **Effects of Thyroid Hormone on the Maturation of the Central Nervous System**
Max Hamburgh *

126. **The Response of Nonanemic Children to the Parenteral Administration of Iron**
Denman Hammond,* Charles McKean,* and Wylda Hammond *

* By Invitation

127. **Total Body Fat in Adolescent Boys**
Felix P. Heald,* Robert Schwartz, Edward E. Hunt Jr.* and Per Helliesen

128. **Tridione Nephrosis in Rats**
Walter Heymann * and Donald B. Hackel *

129. **The Relationship of γ-Globulin Levels to Antibody Response in the Infant**
Ruth Heyn * and Catherine J. Carroll *

130. **Inulin and Free Water Clearance Studies in Potassium-Deficient Rats**
Malcolm Holliday, Thomas Egan,* and Patricia Wirth *

131. **A Functional Enzymatic Deficiency of Cortisol Metabolism in Young Infants**
Gerald H. Holman * and Claude J. Migeon

132. **Fetal Thyroid Development**
Antone G. Jacobson * and Robert L. Brent *

133. **Changes in Acid-Base Balance and Blood Electrolytes in Vigorous and Depressed Newborn Infants**
L. Stanley James *

134. **Study of the Effect of Experimentally Induced Hypothyroidism on Antibody Production, Catabolism, and the Anamnestic Response**
Douglas E. Johnstone and Joe W. Howland *

135. **Experimental Salicylate Poisoning: Effects of a Carbonic Anhydrase Inhibitor and Bicarbonate**
S. A. Kaplan, A. Yuceoglu,* J. Strauss,* and F. T. Del Carmen *

136. **Lactic Acid Milk in Feeding of Premature Infants**
Samuel Karelitz, Herbert I. Goldman, and Norman B. Schell *

137. **Serologic Response to Asian Influenza in Children Following Infection and Vaccination**
D. T. Karzon, R. H. Bussell,* L. Mosovich,* and D. L. King *

138. **Studies of Unesterified Fatty Acid Metabolism in Infants**
Robert Kaye and Mitio Kumagai *

139. **The Plasma Potassium Concentration in Normal Premature Infants**
Hans G. Keitel * and Joycelyn Blakely *

140. **Incidence and Persistence of Poliovirus in the Oropharynx of Children with Acute Poliomyelitis**
Sidney Kibrick, Augusto Schuster,* and John F. Enders *

141. **Blood Volume Studies in Infants and Children as Determined by Cr^{51}**
William B. Kiesewetter,* Kamthorn Sukarochano,* and Lucio Parenzan *

142. **The Effect of Cortisone and ACTH on Chromium^{51} Red Blood Survival in Erythroblastosis Fetalis**
William Krivit *

143. **Electron Microscope Observations on the Human Fetal Adrenal**
Jonathan T. Lanman, Michael H. Ross,* George D. Pappas,* and John Lind *

144. **Familial Pernicious Anemia in Children**
Sanford Leikin *

145. **Effect of Convulsions on the Blood-Cerebrospinal Fluid Barrier and on Cerebrospinal Fluid Levels of Glutamic Oxalacetic Transaminase and Lactic Dehydrogenase**
Miriam Lending *

146. **Studies of Cardiac Rate in Newborn Infants**
Earle L. Lipton * and Julius B. Richmond

147. **The Chloride of Bone**
Charles C. Lobeck *

148. **Observations on Transfer of Vitamin B_{12} from Mother to Fetus and Newborn**
A. Leonard Luhby,* Jack M. Cooperman,* Alvin M. Donnenfeld,* Julia M. Herrero,* David N. Teller,* and Jeffrey B. Wenig *

149. **A Study of the Coagulation Properties of Infants' Platelets**
Arthur E. McElfresh,* Sjarif Thajeb,* and Muzaffer Kurcuoglu *

150. **The Significance of Bone Ages in a Group of Children Referred to an Endocrine Clinic**
William J. Mellman,* Alfred M. Bongiovanni, and John Hope *

151. **Effects of Antimetabolites on Development of the Fetal Rat**
M. Lois Murphy

152. **The Blood-Brain Barrier for Bilirubin During Early Infancy**
Maridee Nasralla,* Eva Gawronska,* and David Yi-Yung Hsia

153. **Maturation of Renal Tubular Functions Demonstrated in Slices of Kidney**
Maria I. New,* Francis T. Kenney,* Helen McNamara,* and Norman Kretchmer

154. **The Intracellular Cation Composition of Skeletal Muscle in Congenital Heart Disease**
Donough O'Brien *

155. **In Vitro Studies of the Effect of Sulfonamides on Bilirubin**
Gerard B. Odell *

* By Invitation

156. Thyroidal Secretion and Degradation Rates in Children
Leo Oliner,* Robert M. Kohlenbrener,* and Ralph Kunstadter *

157. Extrarenal Changes Occurring in Nephrosis During Steroid Diuresis
William J. Oliver * and Charles Sloan *

158. Cinefluorography with Sound: A Technique for the Evaluation of Motor Activity of the Palate and Speech
Richard W. Olmsted * and John A. Kirkpatrick Jr.*

159. A Study of the Effect of High Oxygen Concentrations on the Opossum Pouch Young
Arthur H. Parmelee Jr., Milo B. Brooks,* and Robert S. Stone *

160. An Immunologic Assay for Minute Amounts of Human Pituitary Growth Hormone
Charles H. Read and Daniel B. Stone *

161. Treatment of Idiopathic Female Sex Precocity with Synthetic Progestational Compounds
Robert Richie * and John Crawford

162. An Investigation of Maternal Concerns
William O. Robertson *

163. Clinical Recognition of the Transitional Pulmonary Circulation of Newborns: A Pilot Study Using Auscultation and Electrocardiography
Richard D. Rowe

164. A Study Concerning the Basic Metabolic Defect in Wilson's Disease
Andrew Sass-Kortsak, Robert J. Slater, Douglas G. Geiger,* and Morris Cherniak *

165. Ebstein's Anomaly of the Tricuspid Valve: A Clinical Analysis of Twenty-Four Cases
Gerold L. Schiebler,* Paul Adams Jr., Ray C. Anderson, Kurt Amplatz,* and Richard G. Lester *

166. Studies of Serum Ceruloplasmin During Early Infancy
Julian B. Schorr,* Anatol G. Morell,* and I. Herbert Scheinberg *

167. Glutathione Metabolism in Cord Blood and in the Newborn Infant
A. Sheinberg,* B. Ramot,* A. Adam,* and Ch. Sheba *

168. The Pathogenesis of Proteinuria
R. J. Slater, D. W. Geiger,* and C. P. Rance *

169. Poliovirus Antibody in Cerebrospinal Fluid
Alex J. Steigman, Murray M. Lipton,* and Fe C. Dizon *

170. The Effects of Large Doses of Salicylate on the Metabolism of Cortisol in Human Subjects
Robert Stempfel,* James B. Sidbury, and Claude J. Migeon

171. Angiocardiographic Diagnosis of Aberrant Left Coronary Artery from Pulmonary Artery
Aaron M. Stern,* Norman S. Talner,* and Melvin M. Figley *

172. An Epidermiologic Study of the Nephrotic Syndrome
Gunnar B. Strickler *

173. Hepatic Fibrosis in Children with Acute Leukemia
Charlotte T. C. Tan,* Robert V. P. Hutter,* Frederick H. Shipley,* and M. Lois Murphy

174. The Effect of Immediate Versus Delayed Clamping of the Umbilical Cord on the Venous Pressure of the Newborn Infant
Paul M. Taylor,* Edna L. Birchard,* Nancy H. Bright,* and Thomas J. Egan *

175. Evaluation of Factors Affecting the Intensity of Neonatal Jaundice in Premature Infants
Paul M. Taylor,* Nancy H. Bright,* Edna L. Birchard,* and Doris W. Watson *

176. The Localization and Measure of Phosphorus-32 in Nucleoside Polyphosphates
K. K. Tsuboi *

177. Placental Transport of Labeled Hydrocortisone to Infants with Congenital Adrenal Hypoplasia
Robert A. Ulstrom and Jane Burley *

178. Electrocardiographic Changes in Newborn Premature Infants During Respiratory Distress Syndrome
Robert Usher *

179. Electron Microscopy of Experimental Renal Disease
Robert L. Vernier,* Benjamin Papermaster,* Roger Arhelger,* Fred Mecklenburg,* and Robert A. Good

180. A Preliminary Report on the Use of a New Oral Hypoglycemic Agent in Juvenile Diabetes
W. B. Weil Jr.

181. Failure to Induce Dietary Deficiency of Copper in Premature Infants
J. Wilson * and M. E. Lahey

182. Radioiron Uptake in Iron Deficiency
Calvin W. Woodruff

* By Invitation

183. The Treatment of Glycogen Storage Disease with Glucagon

R. Yim,* J. MacLaughlin,* and S. P. Bessman

Papers Read in Plenary Sessions

1. Disturbance in Tryptophane Metabolism in Phenylketonuria

Heinz Berendes*; J. A. Anderson; M. R. Ziegler,* and Diane Ruttenberg,* Minnesota.

The etiologic relationship between the defect in phenylalanine metabolism to the mental deficiency in phenylketonuric subjects is not completely understood. Determinations of phenylalanine blood levels in 28 phenylketonuric subjects done in this laboratory did not reveal any correlation between the height of the level and the degree of mental retardation. This has been demonstrated by others. Armstrong in 1954 suggested the possibility of a disturbance in tryptophane metabolism in phenylketonuric subjects. Recently Pare and Sandler observed that untreated children with phenylketonuria had reduced urinary excretion of 5-hydroxyindolacetic acid (5-HIAA) and correspondingly reduced blood levels for serotonin. This observation further supports the possibility that a disturbance in tryptophane metabolism is present in phenylketonuria. Serotonin, which is derived through the metabolism of tryptophane, is important because of its role in brain function. The metabolism of tryptophane through serotonin pursues the following pathway: hydroxylation of tryptophane to 5-hydroxytryptophane; decarboxylation to 5-hydroxytryptamine; and finally, oxidative deamination to 5-hydroxyindolacetic acid which is excreted in the urine.

This disturbance in tryptophane metabolism in phenylketonuria was investigated in the following manner. Measurements on the urinary excretion of 5-HIAA were done in phenylketonuric subjects to reflect changes in the metabolism of tryptophane. The following observations were made. The excretion of 5-HIAA was observed in phenylketonuric children on normal diets and during the administration of phenylalanine-low diets. Loading studies were done on phenylketonuric children maintained on normal or phenylalanine-low diets using l-tryptophane and l-phenylalanine and during and following the infusion with 5-hydroxytryptophane.

The results of these studies indicate a reciprocal relationship between the excretion of 5-HIAA and the phenylalanine blood level. The daily excretion of 5-HIAA was lower in untreated phenylketonurics than during the administration of a low-phenylalanine diet. Loading the controlled phenylketonuric child with l-phenylalanine resulted in a decrease in 5-HIAA excretion. Loading with l-tryptophane produced a moderate increase in 5-HIAA excretion. These observations suggest that tryptophane metabolism to 5-HIAA is favorably influenced by reduction in the phenylalanine concentration in the blood.

The metabolic pathway for 5-hydroxytryptophane to 5-HIAA was studied by infusion of large amounts of 5-hydroxytryptophane into uncontrolled phenylketonurics. 5-HIAA excretion rose promptly during and following infusion and was associated with slight improvement in the EEG pattern lasting about 30 minutes. Monoamino oxidase activity was demonstrated in one phenylketonuric child by a decrease in 5-HIAA excretion following the administration of iproniazid.

These preliminary observations permit the following suggestions: there appears to be a competitive inhibition by high levels of phenylalanine for the tryptophane hydroxylase enzyme. The decarboxylation of 5-hydroxytryptophane to serotonin and oxidative deamination to 5-HIAA seems to proceed normally as indicated by an increase in 5-HIAA excretion following 5-hydroxytryptophane infusion.

Discussion

Dr. Samuel P. Bessman, Baltimore: I think that this is an excellent approach, because most investigators have been looking for toxic substances in mental disease. This is a very good indication of the fact that we may have deprivation of substrate in mental disease, and that what we should look for in many cases is what is not there, not what is there in excess.

2. Experiences with a Live Attenuated Measles Virus

Samuel L. Katz*; Donald N. Medearis Jr.,* and John F. Enders,* Harvard.

Introduced by Sidney Kibrick

The Edmonston strain of measles virus, originally isolated in human kidney cell cultures from the blood of a patient in the acute phase of measles, has been passed 24 times in human kidney cells, 28 times in human amnion cells, 6 times in the amniotic sac of the embryonated hen's egg and 25 times in chick cells. In the course of this series of passages there have been changes in the growth curve, titers and cytopathogenic effect of the agent. Material from the 14th chick cell passage has been tested in monkeys to determine if change in virulence and/or antigenicity in vivo has accompanied this altered behavior in vitro.

When administered intravenously and intranasally, or subcutaneously, to susceptible monkeys, the chick cell virus has produced high titers of complement-fixing and neutralizing antibodies against measles virus, but no recognizable disease or viremia. (Early passage human kidney material produced viremia consistently and measles rash

*By invitation

frequently in susceptible monkeys.) Animals immunized subcutaneously 5½ months previously with chick cell virus have been challenegd with the "virulent" human kidney virus and demonstrated a booster response in their complement-fixing and neutralizing antibody levels but no signs of disease or viremia. When early passage virus was given intranasally to these immune monkeys, it was possible to recover the agent from the nasopharynx for 1 to 17 days after inoculation despite continued absence of viremia. Neither the early passage "virulent" nor the late passage "attenuated" virus produced any pathology detectable on sections of brain or spinal cord after intracerebral and intrathecal administration.

In view of these findings of the immunizing capacity of the attenuated virus, a trial in a small group of susceptible children has been planned.

Discussion

Dr. Herbert A. Wenner, Kansas City, Kan.: I would like to compliment Drs. Katz, Medearis, and Enders on this excellent study. However, I have a question, and that is why I am here. I refer to the intracerebral inoculations of monkeys with virus adapted to kidney and chick embryo cultures. I noticed that there were only two monkeys, Dr. Katz, and that the animals were sacrificed after an incubation period of 17 days. What are the reasons for the early sacrifice?

Dr. Samuel L. Katz: Dr. Wenner, first of all, I would like to stress that it is human kidney virus and not monkey kidney virus which we used as our primary strain. This particular strain of virus has never been in monkey kidney tissue. It is all human tissue. As far as the number of monkeys goes, this obviously is not an attempt to assess encephalitis from any sort of statistical point of view. I think you probably feel, as well as we do, that it would take thousands of monkeys inoculated with this virus (if indeed this is the way of producing encephalitis, which perhaps it is not) before you could even approach any sort of ratio comparable to the incidence of encephalitis in human beings. We were only interested in seeing whether direct inoculation of virus into the cerebral tissue or into the spinal fluid would result in any lesion that could be detected. There is not a great deal of evidence to show what does cause encephalitis. I think Drs. Shaffer, Rake and Hodes have a case in the literature with isolation of measles virus through the use of monkeys in one patient with encephalitis. Aside from this, the only other case I have heard of is Dr. Ruckle's in which she allegedly isolated measles virus from the brain of a patient with encephalitis. Whether the presence of virus in the brain causes the encephalitis or not, I do not think anyone is prepared to say. As far as the time at which they were sacrificed is concerned, I agree with you, in a human being with measles your encephalitis may certainly come later than this. In our monkeys, as you saw, the rash occurred from the 9th to the 11th day; add 5 or 6 days to this for the comparable course in human beings; that takes you up to 16 and 17 days. That is why we happened to pick 17 days.

3. Tissue Oxygen Tension During Periods of Extracorporeal Circulation

J. A. Manning*; K. Woodward*; P. V. Gabel,* and S. I. Schwartz,* University of Rochester.
Introduced by G. B. Forbes

The problem of an optimal blood flow rate is critical in the efficient and safe utilization of an extracorporeal system for open heart surgery. This study was designed to define such an optimal rate primarily by a study of tissue oxygen tensions in several organs with associated metabolic and physiologic observations.

The effect of several flow rates on the tissue oxygen tension in four organs was studies and an economic flow rate determined. A combination of the DeWald oxygenator and sigmamotor pump was used to provide an extracorporeal circulation in dogs. By means of a platinum electrode polarograph oxygen tensions were determined simultaneously in the brain, liver, kidney and striated muscle. Control determinations were made subsequent to thoracotomy and insertion of caval and arterial catheters. These findings were compared with the tissue oxygen tensions at fifteen minute intervals for a one hour period with flows of fully saturated blood at 40, 60, 80 and 100 cc/Kgm/min. At flows of 100, 80 and 60 cc/Kgm/min all four organs demonstrated tissue oxygen tensions above the control values with no statistically significant difference between these three flow rates. At 40 cc/Kgm/min the oxygen tension values were either at, or slightly below, the control level and were significantly less than the tensions recorded during the 60 cc/Kgm/min flows.

Paralleling the tissue oxygen tension findings were the blood pressure patterns which showed little alteration between the 100, 80 and 60 cc/-Kgm/min flows but a significant decrease between the 60 and 40 cc/Kgm/min flows. Similarly there was little change between the control and pump values for arteriovenous oxygen difference at flows of 100, 80 and 60 cc/Kgm/min while there was a marked increase in A-V oxygen difference at 40 cc/Kgm/min.

These findings indicate that an economic flow rate can be determined for pumping during the establishment of an extracorporeal circulation. In this experiment this rate was in the range of 60 cc/Kgm/min.

*By invitation

Discussion

DR. SAMUEL KAPLAN, Cincinnati: I should like to comment on the method of measuring tissue oxygen tension with solid cathodes as well as the possible application of the results presented in this study. We have experienced great difficulty in the use of solid polarographic electrodes acutely inserted into tissues just before a reading is desired (Surgical Forum, Philadelphia, W. B. Saunders and Company, pp. 93-96, 1953). These readings are subject to error largely from bleeding around the cathode, movement artefacts, and drifting possibly caused by ischemia at the electrode tip. Because of the instability of the acute electrode, the results should be interpreted very cautiously. It is clear that one of the major functions of a pump oxygenator is to supply at the least the basic oxygen requirements of the patient. It is known that these requirements vary in different age groups and is especially high under the age of two to three years. Dr. Manning's study indicated that during extracorporeal circulation a flow rate of 60 cc/kg/min. was adequate. I believe it would be unwise or even dangerous to apply this figure to the human subject. In clinical practice this flow rate is too low especially in young children. (Extracorporeal Circulation, Springfield, Charles C Thomas, Publisher, pp. 150-163, 1958).

DR. J. A. MANNING: Well, I would say, again, that we found fairly good, or very good, results in the control periods. Until the animal went out, we could, even in the heparinized state, vary the tissue oxygen tension quite precisely by changing the ratio of oxygen in inspired air. Further, the tissue electrodes were placed before heparinization and no significant tissue bleeding occurred. I would certainly agree with Sam Kaplan that to carry these comments to various types of pump oxygenator systems would be totally unwarranted. I think that the interest in our specific experiment was in the various responses of varying organs in this particular preparation to the different flow rates. We would certainly agree that with certain of the other oxygenator systems and different types of pulse pressure curves, different flow rates are indeed necessary.

4. Observations on the Metabolic Effects of Glucagon and Growth Hormone (Human and Beef) in an Infant with Idiopathic Hypoglycemia and Hyperinsulinism

JOHN F. CRIGLER, JR., M.D.; JOHN A. KNAPP, M.D.,* and JEAN CHAGNON, M.D.,* Department of Pediatrics, Harvard Medical School, and the Children's Hospital, Boston.

A four-month-old white female infant was admitted because of convulsions associated with severe hypoglycemia. Initial studies revealed a five- to twenty-five-fold increase in serum insulin-like activity (1000-5000 microunits per milliter) without evidence of pituitary, adrenal, or hepatic abnormality. At five and one-half months of age, after ACTH and cortisone had failed to control satisfactorily the patient's disease, a 66% pancreatectomy with splenectomy was performed. Pathologically the pancreas and liver were normal. Immediately postoperatively, her hypoglycemia returned and again she was found to have an increase in serum insulin-like activity (300 microunits per milliter).

The 70 days of study reported occurred following the first pancreatectomy between the ages of 14 and 16 months. This interval was divided into five therapeutic periods and two periods on no therapy. The therapeutic periods were glucagon alone, glucagon and human growth hormone at two dose levels, human growth hormone, and beef growth hormone. Balance studies in four day periods were carried out continually with the exception of seven days before and after the second pancreatomy.

With 1.8 mg. per day (0.18 mg/kg/day) of glucagon given intramuscularly in divided doses at 4, 2, or 1 hour intervals, a transient rise in blood sugars was obtained. This rise was significant in the morning after overnight fast, but was negligible in the afternoon after feedings at 8:00 a. m. and 12:00 noon. Larger doses of glucagon would produce a response in the afternoon. Human and beef growth hormone had no effect on blood sugars. Following 95% pancreatectomy, blood sugar levels returned to normal.

Balance studies indicated no significant effect on N_2 retention (11.8% dietary N_2 retained) of glucagon at the dose administered. Human growth hormone at 2.0 mg. per day produced a very significant increase in N_2 retention (48.9% retained) which was not maintained by beef growth hormone at 40 mg. per day. In addition, there was a three- to four-fold rise in unesterified fatty acids with human growth hormone, indicating its biological activity.

Studies of serum organic acids revealed a persistent elevation of serum lactate. Pyruvate and citrate levels showed no persistent abnormalities. Plasma epinephrine and arterenol levels were elevated when the hypoglycemia was treated by diet alone and by glucagon administration. During human growth hormone administration and following second pancreatectomy, they were normal. Blood and urinary corticoids were normal or low during the study period. The significance of these findings will be discussed.

*By invitation

Discussion

DR. MARVIN CORNBLATH, Baltimore: We have been studying glucagon effect in newborns, and have found prolonged hyperglycemia in infants delivered after emergency Caesarean section in which the mother has labored. We have not found this prolonged hyperglycemia after elective Caesarean section. We thought perhaps it was related to the 17-hydroxycorticoid levels in these babies. My question is: Did you give glucagon when this patient was on ACTH-gel, and did this have effect in prolonging the hyperglycemia after glucagon?

DR. DONOUGH O'BRIEN, Denver: This seems like an unusual response to glucagon in problems of idiopathic hypoglycemia. I wonder if you had any observations on the glucagon content of the liver by biopsy or other means?

DR. JOHN A. KNAPP: We have quantitative measurements of those levels, but I do not remember the exact values.

DR. ALFRED M. BONGIOVANNI, Philadelphia: I think it would be a mistake to let one of the practical lessons in this very nice paper escape us, namely, that the critical problem is to control hypoglycemia. As you say, the first means did not do the trick. I think this means a number of things. First, it is rare in pediatrics for the surgeon to go in and find a very nice tumor. That, we cannot expect except rarely. I think we must ask the surgeon to do a pretty good subtotal, 90 or 95%, to save the child. Of course those medical means (ACTH, cortisone, etc.) which are so often successful as shown by McQuarrie, should be tried first, but too much time should not be lost if the latter are ineffective.

DR. ROBERT A. ULSTROM, Minneapolis: I would like to congratulate the authors on a very extensive piece of metabolic study. I think the results are very interesting. I may be mistaken, but I would like to put this as a question. I believe the author said he felt there was no diurnal variation in the sugar level of this baby when on treatment. As I looked at the slide, I got the distinct impression there were more of the morning blood sugar levels in the shaded area than there were in the afternoon. I would like to, at least partially, answer Dr. Cornblath's question about the relationships of glucagon and the 17-hydroxycorticoid. This is one of the immediate things that comes to mind on the diurnal variation. It is that children with this type of spontaneous hypoglycemia have diurnal variation. Steroids, eosinophils, etc., have been well shown to have diurnal variation, yet we did not find some years ago any difference in the glucagon effect in adult experiments when given to individuals who were hypoglycemic or normal whether they were getting ACTH or one of the adrenal steroids. We were unable to show any immediate relationship between the two. I would like to ask whether the glucagon used was the crystalline preparation. I would also like to ask whether you have had any experience with this child or others using the preparation that is available in oil. It is said that it can be given at less frequent intervals.

DR. JOHN A. KNAPP: In answer to your question concerning the forms, we used crystalline glucagon. We made an attempt to obtain this other preparation, but our information was that it was not available.

DR. HUTCHINS, San Francisco: I would like to ask how long the human growth hormone was given and whether there was any change in glucose tolerance test during the time? Secondly, were studies done on sodium or calcium during the period of growth hormone administration?

DR. JOHN A. KNAPP: Growth hormone was administered for three 4-day periods. This is human growth hormone. During that time we did not do glucose tolerance tests. We did not do this because we did not want to disturb our balance data. We have done sodium balances, and we are completing phosphate and calcium balances in this child. Concerning the sodium balance, I can say that we found the usual large amount of retention that is found and which you have to be careful when interpreting because of losses through sweat.

DR. JOHN F. CRIGLER JR., Boston: I would like to add one comment in reference to the etiology of this abnormality we are talking about today. We, too, have been very much concerned about the point Dr. Bongiovanni mentioned. There were several things that were not said in the course of the paper due to time. These should be mentioned. As far as the histological status of the pancreas goes, at the first operation, it was entirely normal. On the other hand, the pancreatic sections following the second operation showed definite islet hyperplasia. This is an interesting finding in light of persistent hypoglycemia and poses the question that we have been asking ourselves: What is the stimulation to islet hyperplasia in this patient with persistent severe hypoglycemia? Another point that may be of interest to everyone is the fact that during the course of these studies, insulin tolerance tests revealed that the patient was quite sensitive to insulin even in the presence of increased insulin-like activity in her serum as determined by assay. This has also been found since her blood sugar has been in the normal range, after the second pancreatectomy. In addition, the sensitivity of patients of this type to certain amino acids, as reported by Cochrane, Payne et al., was present in this child also. All of these points make us wonder whether the primary defect in this disease is in the pancreas or in the peripheral cells or in the central nervous system or in some other place. Part of

the reason for doing these studies was to try to shed some light on the etiology of this very complex disease. We have been unsuccessful, unfortunately, in relieving hypoglycemia in some of these patients even following surgery. I gather this has been a rather general experience. It is obvious that if we are radical enough in removing the pancreas, we should be able to correct the hypoglycemia. However, I wonder if we correct the basic defect.

5. Osmotic Characteristics of Renal Papillae and Urine in Relation to Counter-Current Loop Schemes of Concentration of Urine

J. W. Th. Appelboom,* and William A. Brodsky, Louisville

If concentration of urine occurs in collecting ducts by equilibration of their fluid with an osmotic gradient formed in a counter-current loop of vasa recta or of tubule, then the osmotic activity of a distal part of the renal papillary tissue could be equal to or greater than that of urine. However, the measured osmotic activity of canine renal papilla was invariably less than that of hypertonic urine over a wide range of urinary osmotic activities—300 to 1000 mOsm/L. These data require the invention of certain ad hoc postulates in order to maintain the validity of the various counter-current schemes of urinary concentration.

The kidneys of anesthetized dogs excreting urine at steady state levels of osmotic activity were excised, and cut so that the papillae were separated from the medullae and cortices in 5-10 minutes. Papillae were weighed quickly, and immersed in boiling water for 1-2 hours. Estimates of osmotic activity of the original undiluted tissue were made from freezing point determinations of the supernatant, and from data on weight of tissue and added water. (Appelboom, J. W.: *Fed. Proc.* 16: 278, 1957). Representative data in one experiment were: Osmotic activity of urine=600 mOsm/L; of papillary tissue=450; of blood=300; and of renal cortex, 300 mOsm/L. Papillary tissue (without urine) could be isotonic with plasma, if urine constituted *ca.* 50% of the volume of the whole tissue. A ratio of urine volume/tissue volume of *ca.* 0.5 was confirmed by two histological techniques, and by calculation from dry weight data on papillae and urine.

If osmotic gradients really exist in counter-current loops of blood vessels and tubules, they must be restricted to the distal 0.5 cm. of papillary tips. This, in turn, would demand a gradient of dry weight of papillary cells. On the other hand, if renal papillary cells and interstitial fluid were isotonic to plasma, one could account for data on osmotic activity and on dry weight with no ad hoc postulates.

*By invitation

Discussion

Dr. Wallace W. McCrory, Philadelphia: I would like to express my appreciation to the authors for an arduous project very understandably presented on a complicated subject. I have one question. I noted that you were unable to account for the total osmotic activity by the contribution of electrolytes. Did you measure the urea concentration in the papillae to see if this would account for the difference?

Dr. William A. Brodsky: No, but we assume it would.

Dr. Robert W. Winters, Chapel Hill, N. C.: I would like to ask Dr. Brodsky another question about urea. Would it be possible to have a large amount of urea diffuse from the collecting duct into the loop and account for the high osmolarity on the tip of the papillae of the loop? Then, when the urine comes up into the distal tubule, would it be possible for urea diffuse out again?

Dr. William A. Brodsky: Do you mean that urea diffuses from the interstitial fluid into the loop of Henle and renders the lumen fluid hypertonic? Urea could diffuse from an hypertonic interstitial fluid into the loop and render the loop fluid hypertonic. In the Berliner scheme, the loop walls are "water-tight." If this scheme is valid, and if a positive answer to your question is also valid, this would mean that loop walls are freely permeable to urea and impermeable to water. However, the current picture is that urea (from a hypertonic interstitial fluid) diffuses into the lumen of the collecting duct while water moves from collecting duct to interstitial fluid, i. e., water moves in a direction predicted by the osmotic force across a membrane permeable to water.

Dr. Ralph Wedgwood, Cleveland: I would like to ask Dr. Brodsky whether any artifact was introduced by boiling and diluting the tissue prior to the measurement of osmotic concentration?

Dr. William A. Brodsky: We checked this in a tedious manner for about a year, and I can summarize in this way; the artifacts are all of the order of 1 to 3%. In this field, errors of 10 or 20 milliosmols out of 300 are not bad ones. Moreover, results with an independent method by Leaf and Mattingly have confirmed our data on the isotonic boiled tissue. We were delighted to see that the process of boiling and diluting yielded the same answer as did a process of freezing to solid state and melting.

6. On the Origin of Variants of Human Amniotic Cells in Tissue Culture

JUAN C. RIVADENEIRA * and FREDERICK C. ROBBINS,
Cuyahoga County Hospital and Western Reserve

In the past 15 years various investigators have observed the appearance of changes in the morphologic characteristics of mammalian cells maintained in vitro. This phenomenon has been observed to occur in a variety of tissues of different origins. In some instances, such altered cell lines were found to produce tumors in animals, a property thought to be peculiar of neoplastic cells.

Since 1955, human amniotic membranes have been extensively used in our laboratory as a source of cells for virus studies. On three occasions abnormal cells have appeared in primary cultures of these cells. The first variant strain of amniotic cells (AV-1) arose in cultures of AC which had been inoculated with specimens from a case of rubella. The second strain (AV-2) originated in tubes of AC that had received as inoculum material from a case of infectious hepatitis. The third strain of AV cells (AV-3) appeared in a bottle of amniotic cells which had not received inoculum of any kind.

In the first two instances, the uninoculated control tubes of AC were observed for six weeks and showed no changes. In contrast to previously reported alterations in normal cells, which occurred during attempts at continuous cultivation, the strains of AV cells appeared in primary cultures of amniotic cells when the cultures were 15 to 20 days old. Microscopically, these cells appeared as collections of small hyperchromatic cells showing an array of morphologic aberrations, such as altered cytoplasm-nucleus ratio, increased number of nucleoli, great variation in size and shape of nuclei, and numerous abnormal mitosis. They also showed an increased rate of proliferation and unlike the parent tissue they were readily established in continuous culture. Attempts to reproduce the phenomenon by inoculation of new cultures of AC with the original human specimens, and by other means, failed. The virus sensitivities of AV cells show some interesting differences when compared with that of "normal" amniotic cells. In addition, the transplantability in vivo of AC cells also differs from that of amniotic cells.

Discussion

DR. RICHARD T. SMITH, Dallas: I would like to ask what may be a very obvious and naive question to virologists. Is it possible that the amnions used could be contaminated by other cells from the placenta from which they were obtained?

DR. SAMUEL L. KATZ, Boston: I would like to ask Dr. Robbins if he has any data in relation to the origin of these cells. By that, I mean they all come from placentas, obviously; probably from babies that have been delivered vaginally. However, we have had some interesting variations in virus susceptibility of primary amnion cultures. In trying to track down the possible factors involved, there seems to be some difference based on the sex of the baby from which the amnion was derived. More specifically, female cells have been more susceptible in some instances to certain viruses than amnion cultures derived from placentas which nurtured male babies. There seem to have been other factors. I wonder if there is any background data for these three strains of cells you have described as far as the sex of the baby from which they originated is concerned. Is there any question of radiation or x-ray pelvimetry to the mothers of the babies prior to delivery?

DR. M. MICHAEL SIGEL, Miami, Fla.: We have no data to confirm this particular observation in tissue cultures. However, in one experiment in vivo in which amnion was administered to conditioned rats, we did note development of tumor, and, according to the pathologist, the occurrence of cancer-like cells. This was a primary human amnion transplanted, or rather injected, into rats. Perhaps in this instance also, malignant cells may have been present in the original tissue or may have arisen without in vitro passage.

DR. FREDERICK C. ROBBINS: In answer to Dr. Smith, we were concerned about the possibility that other cells from the placenta might have contaminated our cultures. We deliberately added chorionic and other cells to see if we could reproduce this phenomenon but were unable to do so. In answer to Dr. Katz, we were more concerned as to whether or not the mother had any history of malignancy or other disease. We never thought of asking about the sex of the child. As far as we can make out from our investigation of the mothers, they were normal, young, and multiparous, and had received no irradiation or other treatment. One thing that we cannot completely control is the possibility of mutagenic substances being introduced during the preparation of the mother before delivery. We request that nothing be used except soap and water, but this may not always happen. Dr. Sigel, we used cultured amnion cells that appeared normal as our control material for injection of rats. These produced no tumors. There was a distinct difference between the behavior of the rats inoculated with normal amnion cells and those with the variants, even though I would hardly call the tumor produced by the variant cells a malignant lesion. Thank you.

*By invitation

7. Preliminary Observations with a Procedure for Defining Human Factors of Importance in the Medical Management of Children with Long-Term Illnesses

STUART FLERLAGE*; MARILYN BAILEY,* and NATHAN B. TALBOT, Harvard

It has been our experience that a considerable number of patients with enduring metabolic or physical handicaps may fail to do well because they do not take full advantage of the medical therapy and other opportunities potentially available to them. The present studies were undertaken in an effort to find out why this occurs and what the physician can do to ameliorate this problem. This paper summarizes the results obtained on four representative children and young adolescents suffering, respectively, from asthma, obesity, anorexia nervosa and osteogenesis imperfecta.

The studies consisted first in a definition of the effects upon the patients' daily life of (*a*) the disease itself, (*b*) the therapy prescribed by the physician, (*c*) the limitations imposed on the child's family and (*d*) the restrictions imposed by the patient himself because of his condition. The information obtained has been set forth in bar graphs which indicate in approximate terms what portion of the child's sleeping, eating, self-care, schooling, working and free time activities are influenced by each of these factors. In some instances it was found that the disease was acting directly or indirectly to disturb essentially all of the child's normal daily activities.

Secondly, attention has been given to each patient's human ecologic status because it was noted that chronically handicapped patients who are doing well usually are in contact with people who care about them in a nonpossessive, understanding manner and are contributing to others in a manner appropriate for their age. This "balance" of social intake and output also has been recorded in a simplified diagrammatic manner. Almost without exception this "balance" has been found to be grossly disturbed in patients whose progress has been unsatisfactory.

Thirdly, as the patient, his family, and the physician have become aware of the information outlined above, there has been a tendency to modify the therapeutic regimen in such a manner as to minimize the effects of the primary disease upon the patient's life and to take the steps needed to provide a satisfactory "social balance." This has been followed in several instances by improvement in the patient's condition.

It is concluded that the direct and indirect effects of enduring physical and metabolic handicaps upon a child's life are apt to be unnecessarily great unless steps are taken to define and to minimize them.

*By invitation

Discussion

DR. JULIUS B. RICHMOND, Syracuse, N. Y.: I would like to congratulate the authors of this paper for making a very serious attempt to quantify the factors which are obviously difficult to quantify. Yet, all of us face such problems in our daily clinical practice. We have need for a quantitative approach to the study of what Dr. Alan Gregg referred to as multifactorial concepts of disease. I think, however, it should be pointed out that in this grouping of disorders, there is a mixing of some problems which are, to a very considerable extent, psychological in origin with social and psychological problems which are secondary to physical disease. These are very different kinds of disorders; mixing them is akin to mixing apples and oranges. It seems to me that it would be unfortunate if one gained from this presentation the impression that some of the more formidable psychiatric disorders would be very amenable to the manipulative kind of approach presented very briefly in this study. I would like to know, therefore, whether in relationship to such problems as, for example, the asthmatic child who was brought into bed with the parents because of their anxiety in relationship to the child's undernutrition, a situation obviously laden with psychological problems, whether any individual psychotherapeutic efforts were undertaken.

DR. STUART FLERLAGE: In reply to Dr. Richmond, no attempts have been made to treat children with primary psychopathology. We have restricted ourselves only to those presented with somatic complaints. Secondly, in regard to the child with asthma, who lived in a country town about 150 miles north of Boston, psychotherapy had been attempted by the pediatrician in conjunction with the local Child Guidance Clinic, one which traveled around through various small towns in New Hampshire. This had failed previous to her being sent here. The parents would not cooperate, and the child had not been seen. Incidentally, the girl is off cortisone. Secondly, in the child with caloric undernutrition, no attempt at psychotherapy with the parents or the child has been made. We recognize, however, the fact that there may be factors beyond the scope of this method at present.

8. Studies on the Hereditary Enzymatic Defect in Certain Drug-Induced Hemolytic Anemias

RUTH T. GROSS; PAUL A. MARKS*; RUTH E. HURWITZ*; JUDITH BEASLEY,* and MOYRA SUI,* Stanford and Columbia
Stanford and Columbia

Hemolytic anemia induced by ingestion of certain chemical agents has been related to abnormalities in erythrocyte (RBC) metabolism. Several investigators, measuring RBC glutathione have

found that a hereditary defect is common to these anemias.

Carson demonstrated a deficiency in glucose-6-phosphate dehydrogenase (G-6-PD) in subjects sensitive to primaquine. G-6-PD catalyzes the oxidation of glucose-6-phosphate resulting in generation of reduced triphosphopyridine nucleotide.

This study demonstrates that a hereditary deficiency of erythrocyte G-6-PD is an essential lesion in these drug induced hemolytic anemias.

In a group of 400 healthy subjects without a history of hemolytic anemia, G-6-PD deficiency was present in 10% of the Negroes and 2% of the Caucasians. This enzyme deficiency was also detected in subjects known to have had hemolytic anemia, 5 from ingestion of naphthaline, 4 from fava beans and 3 from unknown causes.

Among 100 relatives of the subjects with low enzymes, 40% were found to be deficient. In 23 instances complete families were studied. The data obtained establish the hereditary nature of the enzymatic defect and support the theory that the gene is sex-linked.

Subjects with deficiency of G-6-PD fall into two categories, one with very low enzyme levels and the other with intermediate levels. The sex distribution of the subjects in these two categories further elucidates the mode of inheritance of the gene.

A better understanding of the physiological and genetic significance of the two levels of enzyme activity is being sought. Studies will be presented which compare the activity of G-6-PD in old and young cells in subjects with varying levels of enzyme.

Discussion

Dr. Paul R. Patterson, Albany, N. Y.: I just wish to say that I think this is one of the finest prepared and presented papers that I have heard. I think it is a good example for all of us. [Applause]

Dr. Gross: Perhaps this would be a good time to stop and comment.

Dr. Joseph Dancis, New York: I wonder whether the sex modification might be related to estrogen activity and its effect on oxidation-reduction reactions.

Dr. Samuel Wishik, Pittsburgh: Is your finding of a predominance of low levels in males confirmed by clinical experience? It might be expected that more of the accidental drug induced hemolytic anemias occur in males.

Dr. Jean Dawson, Boston: We have had some experience in cases like this. We have one male patient whom we think fits into the intermediate pattern rather than the severe. His mother is intermediate, and his father is normal. We have been able to follow a woman who was known to be glutathione unstable. During this period she developed a hemolytic anemia of unknown etiology, characterized by mild anemia and reticulocytosis. At this time her glutathione stability test and glucose-6-phosphate dehydrogenase activity became normal. This would confirm your findings on increased levels of enzyme activity in reticulocytes. We also had an incident of hemolysis in patients with the intermediate form of the disease. In fact, most of our patients are intermediates and females.

Dr. Ruth T. Gross: First of all, many thanks to Dr. Patterson. I cannot answer Dr. Dancis' question about estrogen activities with certainty, but I think it is extremely likely that this could be a factor in the sex modification. In answer to Dr. Wishik's question, in our experience, most of the individuals who have undergone hemolysis have been males, and this is true in the literature as well, particularly in the Italian and Israeli literature, where so much experience has been gained from acute episodes of Favism. In answer to Dr. Dawson's questions, we too have found one intermediate male only. This person did not experience hemolytic anemia. I am very gratified to hear that the enzyme levels rose during the hemolytic episodes in your patients. This affords me the opportunity to make one more comment, if I may, on the last slide which I showed you. The fact that the low enzyme individuals can produce a fairly acceptable enzyme level in their youngest cells may, at least partially, explain another clinically interesting fact, namely, that when a person has experienced a hemolytic crisis, he is then refractory to further hemolysis, even though he continues to ingest the same agent. Perhaps, this is the result of enrichment of the erythrocyte population with young cells containing high levels of enzymes.

9. Cyanosis Due to an Abnormal Hemoglobin

Park S. Gerald* and Louis K. Diamond, Harvard

A child presenting with complaints attributable to a peripheral neuritis was found to be mildly cyanotic as well. The patient's father was similarly cyanotic and by history the condition had been present in at least the two preceding generations. Upon examination of the father's venous blood it was discovered that the abnormal color could not be altered by shaking in air nor could methemoglobin be detected by the conventional (Evelyn and Malloy) spectroscopic test.

By means of electrophoresis the father's hemoglobin could be separated into a normal and an abnormal fraction. The abnormal hemoglobin, designated Hgb M, was found to be unique in that its methemoglobin form had an abnormal spectral curve. Unlike normal methemoglobin, the methemo-

*By invitation

globin form of Hgb M does not react completely with agents such as cyanide. On the basis of this abnormal reactivity it is possible to explain (1) the cyanosis as due to the presence of methemoglobin M which the normal erythrocyte enzyme systems are unable to reduce and (2) the failure of such agents as ascorbic acid to relieve the methemoglobinemic cyanosis for the same reason. The dominant inheritance of the cyanosis is presumed to indicate a similar mode of transmission of the abnormal hemoglobin.

Two forms of familial methemoglobinemic cyanosis are thus now recognizable; (*a*) that transmitted as a recessive disorder and due to an abnormality of the erythrocyte enzyme systems, and (*b*) that transmitted as a dominant characteristic and associated with the presence of Hgb M.

Discussion

DR. IRVING SCHULMAN, New York: This is an extremely interesting paper. I would like to ask two questions. Does the presence of this hemoglobin lead to anemia in the affected individuals? Secondly, do you place any significance on the peripheral neuritis which is described in the first sentence of your abstract?

DR. JEAN DAWSON, Boston: I wonder if any in vitro studies of the red cell function in regard to enzyme were carried out, such as incubation with glucose and methylene blue?

DR. ARTHUR E. MCELFRESH, Philadelphia: I would like to ask whether there has been any determination of the isoelectric point of this hemoglobin and whether this must not also be an abnormal since it can be separated by electrophoresis or resin.

DR. PARK S. GERALD, Boston: Let me begin with the last question. It was noted that the hemoglobin had been demonstrated to be abnormal in its globin fraction by the original German workers, and we are quite in agreement with this. Study of the alkaline and acid hemochromogens by the German workers indicated that no abnormality of these derivates was present. We have likewise investigated the alkaline hemochromogen and find it to be normal. It is presumed, therefore, that the heme group is perfectly normal and that the abnormality resides in the globin, the nature of which is too complicated to illustrate at this time. As to Dr. Dawson's comments about enzyme activity, we did not make any such studies. The German workers have done extensive studies of this nature and did not detect any deficiency in activity. To Dr. Schulman, we have no evidence of any hematologic abnormality in this patient other than the obvious consequence of an abnormally colored pigment. He did not have polycythemia, abnormal red cells, or alteration of the reticulocyte count. It is of interest, however, that Dr. Pisciotta has reported a patient in which Hgb M in combination with another abnormal hemoglobin, as yet unidentified, gave a hemolytic anemia. Lastly, regarding your comment about peripheral neuritis, this is the only affected member of this pedigree with peripheral neuritis. We have since identified a second pedigree of Hgb M disease which has not possessed any neurologic abnormality of this nature. We presume that it is a coincidental finding only.

10. The "Stuffy Nose Syndrome" of Premature Infants; An Example of Bacterial-Viral Synergism

HEINZ F. EICHENWALD, Cornell

As part of a systematic study of the effects of viral infections on premature infants, the "stuffy nose syndrome" was investigated from a microbiologic and epidemiologic standpoint. The features of this syndrome are a stuffy nose with intermittent or continuous mucoid nasal discharge, without systemic manifestations of infection. On close inspection, moderate hyperemia of the nasal and pharyngeal mucosa is found, with mild hyperplasia of the pharyngeal lymph tissue.

The condition persists usually for a week or more, and rarely, if ever, progresses to more serious respiratory or systemic illness.

Once this disease has been introduced into a nursery, efforts to eradicate it are usually unsuccessful, and 20% to 40% of infants admitted to an infected unit will show these manifestations at some time during their stay.

Epidemiologic and microbiologic investigations indicate that the "stuffy nose syndrome" represents an example of bacterial-viral synergism in the pathogenesis of disease. From an initial series of thirty infants with stuffy noses, adenovirus type 1 was isolated from all, along with a coagulase positive hemolytic staphylococcus aureus. Twenty-two other infants who remained well harbored the same virus but no staphylococci, while forty-one others, also healthy, showed the staphylococcus in their nasopharynx, but no virus was isolated.

In another nursery outbreak of the "stuffy nose syndrome," an entirely different virus was isolated which also appeared to be acting synergistically with a staphylococcus. These two outbreaks will be described and additional evidence favoring the concept of a bacterial-viral synergism will be presented.

Discussion

DR. REICHELDERFER, Bethesda, Md.: We had an opportunity last fall of studying in the Washington, D. C. General Hospital an epidemic of stuffy noses as described by Dr. Eichenwald. We were unable to isolate any virus in kidney tissue culture or any

bacteria. I would like to ask Dr. Eichenwald if he was able to demonstrate an antibody response in these premature infants. We were unable to do so with this group of infants.

DR. CLEMENT A. SMITH, Boston: Dr. Eichenwald did not tell us whether this occurred in the adult personnel, especially the nurses in the nursery. I am sure that we would like an information on that also.

DR. HORACE L. HODES, New York: Dr. Ralph Moloshok called my attention to this syndrome sometime ago, and I think he noticed the onset of this condition when the infants were removed from the isolette. I wonder whether the humidity to which the baby was subjected had anything to do with the incidence of the symptoms in the individual cases and whether, epidemiologically, this could be related to the disease syndrome.

DR. HEINZ F. EICHENWALD: I would like to answer the first question on antibody response in premature infants in detail, but time will not permit this. I might briefly summarize our observations on antibody production in premature infants as follows: whether or not an infant responds to an infection with a given viral agent by forming antibodies appears to depend not so much on the maturity of the baby, but rather on the particular virus involved. We have studied a number of different types of viral infection in premature infants. There are some viruses, particularly ECHO 18 which almost invariably result in antibody production in premature infants. On the other hand, with certain other agents, particularly the adenoviruses, antibodies are not usually demonstrable after infection. Whether stuffy noses in adults and in older infants and children are produced by a similar combination of events is something I do not know. It is difficult to study a problem of this type in open population groups. Premature infants are ideal. They are isolated from outside contacts and stay in the hospital for fairly long periods of time. Therefore, they lend themselves to detailed epidemologic as well as laboratory investigation. The third question asked was whether the humidity in the incubators might predispose the infants to stuffy noses. I would say no, as far as the epidemic type of stuffy nose is concerned. You may recall that one of the slides showed that the incubator babies actually had a lower incidence of this condition than those maintained in bassinets. As I am sure you have observed, there exists also a sporadic type of stuffy nose; we have not investigated the etiology of this. Here humidity may play an important role.

11. Mechanisms in Infantile Acrodynia (Pink Disease)

DONALD B. CHEEK, Dallas, Texas

Mercury is a factor in the etiology of acrodynia. The mode of action is unknown. Sweating, hypertension, tachycardia, vasospasm, and hypotonia have been regarded by some workers as evidence of sympathetic hyperactivity. Our more recent findings of raised insensible water loss (B. M. R. raised), increased venous pressure, and raised urine steroid excretion, also could arise from epinephrine activity. Changes in fluid physiology from previous work include loss of E. C. V., hemoconcentration, raised sweat-water loss with minimal urine volume, tendency to low serum Na concentration, low sweat Na concentration, achlorhydria, and a failure to restore extracellular NaCl losses. The problem is to relate all these findings to the effects of mercury.

Rat experiments suggest that calomel can affect the tissue response to epinephrine and can produce alteration in growth and body composition. Repeated doses of calomel cause loss of tissue NaCl and a slight increase in body K. These changes are seen with or without functional renal insufficiency (Acidosis, high BUN). Mercuhydrin, for contrast, produces similar losses of tissue NaCl. In young rats (80 to 100 gm.), if 10 mg. of calomel is given with 0.5 mg. of epinephrine in oil and drinking water is allowed, the rats develop within 12 to 22 hours a peculiar cold feeling, weakness, a scrawny appearance, cyanosis in their extremities in some instances, increased insensible water loss, hemoconcentration, a 16% reduction of total chloride, slightly raised BUN, and hypertension which is sustained. Tubular necrosis appears in the outer medulla of the kidney. Control rats given epinephrine only, do show hypertension, loss of chloride and increased insensible water loss but to a lesser extent. Hypertension is not sustained. Young rats given 5 to 10 mg. of calomel and 1μg. of epinephrine intravenously, immediately show a decided increase in pulse rate. Control rats do not. It is tentatively concluded that calomel can alter, possibly enhance, the tissue response to epinephrine and can produce losses of body NaCl.

In acrodynia it is suggested as a working hypothesis that ingestion of mercury leads to renal accumulation of mercury and salt loss. In addition mercury may enhance the tissue response to epinephrine which significantly increases skin water losses. The end result of renal salt and skin water loss is hemoconcentration and loss of E. C. V. This loss of volume stimulates the adrenal gland, cortex and medulla. Perhaps salt active steroids cannot counter, however, the predominant renal natriuretic effects of mercury. Adrenal medullary stimulation would lead to continued sympathetic activity, vasospasm, etc., with probable renal anoxia and liberation of renal pressor factors. Renal damage is likely to coexist and ensure further vasospasm. It is assumed that mercury concomitantly inhibits enzymatic activity (SH groups), and our previous finding of oxyhemoglobin in the

plasma (hemoglobin is known to cause vasospasm) may be related to enzyme factors.

Discussion

DR. ROBERT COOKE, Baltimore: I wonder whether Dr. Cheek feels that the quantities of mercury absorbed in infants from teething powder are comparable to the doses he had administered to his rats. This would be about a gram to a 10 kg. baby. The other question is whether or not the administration of BAL at the same time as epinephrine and mercury prevented the physiologic and chemical changes.

DR. DONALD B. CHEEK: I thank Dr. Cooke for the comment. In respect to the amounts of calomel, it is true, of course, that these rats on a weight basis were receiving more calomel than many patients with acrodynia have been thought to have received. However, I feel that this is a difficult question to answer because it involves the whole problem of mercury absorption. It is definitely known that the degree of absorption of calomel depends upon the alkalinity of the intestine. If the pH of the intestine is high, large amounts of mercury will be absorbed, from a given dose. There is no question about the fact that calomel is effectively absorbed from the intestines in experimental animals; Barrett of Sydney has shown that as much as 40% of the mercury can be absorbed from a given dose of calomel. As much mercury is also absorbed from an equal dose of mercuric acetate, a certified poison. In acrodynia, on occasion, there have been cases reported which have had much larger amounts of calomel than I have administered to these rats. Some children have had repeated doses of mercury (skin ointments) over long periods. It may be that much smaller doses of calomel would produce the results that I have shown in these rats and coincidental sympathetic stimulation may be a more important factor. I have not used BAL in these experiments to see what the effect would be. Therefore, I cannot answer the latter part of your question.

12. Observations on the Control of Bilirubinemia

STUART DANOFF*; AUDREY BOYER,* and L. EMMETT HOLT JR., New York University

Indirect bilirubin is now commonly regarded as a neurotoxic substance; it is thought to be the most important, if not the sole factor responsible for kernicterus, and strenuous efforts are made to keep its concentration below 20 mgm. % in the newborn infant. Direct bilirubin, on the other hand, a glucuronic acid conjugate of the indirect reacting pigment, appears to be non-toxic to the brain and is, moreover, readily excreted in the urine. Any measure that would promote glucuronidation, converting indirect to direct bilirubin would have obvious therapeutic applications.

The natural pathway of glucuronide formation, Glucose→Uridine diphospho glucose→Uridine diphospho glucuronic acid→X glucuronide, appears to be imperfectly developed in the newborn infant and efforts to push this by adding intermediates would seem to be impractical, particularly since the block is known to be in the last step of reaction —a defect of the enzyme glucuronyl transferase which transfers the glucuronic acid from the uridine diphosphate to the substance to be conjugated.

It is, however, quite conceivable that other pathways of glucuronidation exists. The possibility that glucuronidation could be promoted by the direct administration of glucuronic acid has attracted a number of investigators, but the evidence on this point is conflicting. Douglas and King, using C^{14} labelled glucuronic acid in borneol fed rats, recovered from 2% to 6% of the tagged material as borneol glucuronide. On the other hand, Eisenberg, Field, and Stetten were unable to confirm this finding in two human subjects, one of whom was given C^{13} glucuronic acid and the other C^{13} glucuronolactone. Since bilirubin is conjugated somewhat differently from borneol (ester linkage rather than glucoside linkage) and particularly because of the small number of observations in man, it seemed desirable to attempt the conjugation of indirect bilirubin in the newborn infant by the administration of glucuronic acid. This attempt has met with a considerable measure of success. In 16 out of 27 cases of hyperbilirubinemia of the newborn (due to erythroblastosis, prematurity or unusually severe physiological icterus in full-term infants) the oral administration of glucuronic acid resulted in a sharp drop of the indirect bilirubin of the serum. This was accompanied in some instances by a simultaneous but small rise in the direct bilirubin concentration. Because of irregular absorption of glucuronic acid and because of complicating diarrhea and acidosis which sometimes resulted from its oral use we now prefer to give it intravenously. By this means we have been able to control the indirect bilirubinemia in eight out of ten consecutive cases.

Discussion

DR. RICHARD L. DAY, Brooklyn: Since therapeutic recommendations were made in this paper, I would like to mention an interesting fact which has been uncovered by Dr. Lois Johnson. She will be presenting this material tonight, along with other material on the subject. The genetically jaundiced rats whose livers are deficient in glucuronyl trans-

* By Invitation

ferase and which develop kernicterus have a depression in serum bilirubin when given glucuronate, about as often and to about the same extent as Dr. Danoff has reported. Unfortunately, these young rats are made much sicker by this material, and the sickness is kernicterus. Normal, nonicteric rats are unharmed by the drug. An alternative hypothesis to that of conjugation can, therefore, be entertained; the bilirubin might be shifted among body compartments. The observations that Dr. Johnson has made with sodium chloride and sulfa drugs parallel those of glucuronate injection, and seem best explained by such a theory, though just where the bilirubin disappears to remains, so far as I know, undetermined.

Dr. David Hsia, Chicago: We have had the opportunity of using C^{14}-labeled sodium glucuronate administered intravenously to adult dogs at the rate of 100 mg/lb/hr., which is the same dose as recommended here. A total of 240 mg. of indirect bilirubin was then injected intravenously, which resulted in a serum bilirubin level of 10 mg. % in the animal. Over a four hour period, approximately half of the injected bilirubin was recovered through the bile collected by inserting a cannula into the common duct. This bile was separated by paper chromatography, according to the method of Schmid. No significant activity could be detected either in bilirubin or in the bile itself. On the other hand, 84% of the injected radioactive material was recovered in the urine samples free of bile which were collected over the same period. Although these experiments were done in an adult animal, there is no great evidence that sodium glucuronate is converted to bilirubin glucuronide.

Dr. Rudi Schmid, Boston: We have been exceedingly interested in Dr. Danoff's report. Since we first heard of the results of Dr. Holt and his co-workers last November, we have treated a total of nine infants with glucuronic acid. These studies were done at the Boston City Hospital in collaboration with Drs. Gloria Jeliu and Sidney Gellis. Maybe we could have one of our two slides to show the results. Four infants were given 2-3 gm. of glucuronic acid by mouth every hour for four hours. As you can see, we did not observe a significant change in the serum bilirubin concentration. After we had this negative experience, we began to use sodium glucuronide by intravenous route. The first three children received 30 to 42 mg/kg/hr. for several hours, making a total dose of 150 or 250 mg. per baby. You can see that there was no significant change in serum bilirubin. We then increased the dose to 200 and 250 mg/kg/hr., which makes a total dose of between 3 and 5 gm. for a 2 kg. baby. With this almost heroic dose, we did not find a significant change in serum bilirubin. In one of the curves, a temporary fall from 19 to 17 mg. % was observed, which can hardly be considered significant. This morning, at the Association of American Physicians, Dr. Holt implied that differences in techniques may account for our failure to repeat his results. This prompts me to say that Dr. Danoff has been kind enough to visit Boston and to supervise personally the treatment of Infant No. 9. He has decided how much glucuronic acid to give, in which form and for how long. As can be seen, no change in bilirubin concentration was achieved. One may argue, I assume, that our group of treated infants was rather small and that had we continued with our efforts, we may ultimately have succeeded. On the other hand, we hesitated to do so because of the following considerations: We feel that there is no convincing evidence which would suggest that glucuronic acid is really used for conjugation of bilirubin. The available information regarding mechanisms by which glucuronides are formed suggests that glucuronic acid is not used directly but has to be derived from phosphorylated glucose. We feel, therefore, that there is no biochemical basis for the assumption that glucuronic acid is used for the formation of bilirubin glucuronide. However, if one attempts to find a possible explanation for Dr. Danoff's observations, one has to consider the possibility that such large amounts of glucuronic acid may result in a redistribution of bilirubin between the vascular space and the tissues and that the glucuronic acid, so to speak, may push the pigment temporarily into the organs. We feel that this potential hazard to the baby should be definitely ruled out before neonatal jaundice is treated with glucuronic acid. Thank you.

Dr. Gerard B. Odell, Baltimore: We have been studying the protein binding of bilirubin and have found that certain organic anions, particularly sulfonamides and salicylate, will displace bilirubin from its protein binding, making it more diffusible. This can be demonstrated by ultrafiltration and/or dialysis of serum containing high concentrations of bilirubin. When we heard of Dr. Holt's observations, we naturally wondered if glucuronic acid had a similar effect. However, we have been unable to demonstrate any interference by glucuronic acid on the protein-bound bilirubin. We have also tested various concentrations of glucuronic acid for its possible effect on the van den Berg reaction and have found no interference. As a possible explanation for the decrease in bilirubin concentration observed by Dr. Holt's group, I suspect some of this may be due to simple dilution. Both glucuronic acid and sodium remain in the extracellular fluid and, if not excreted as fast as it is given, would increase the osmolarity of the extracellular space significantly causing movement of water from the intracellular to the extracellular space. If the

sodium glucuronate were given as an isotonic solution, this would also cause a considerable dilution if the solution were not excreted as rapidly as given because of the amount of fluid needed to give 10-15 gm. of sodium glucuronate isotonically.

DR. WILLIAM J. WATERS, Syracuse, N. Y.: I would like to ask Dr. Danoff if he has any measurements in the urine or stool to show an increased excretion of bilirubin. Secondly, in conjunction with what Dr. Schmid has said, we had one experience with an infant whose bilirubin rose to 25 mg. % at the age of about 30 hours on the basis of an Rh incompatibility. We administered 5 gm. of glucuronic acid intravenously over a four hour period. We took bilirubin determinations at hourly intervals. During this four hour observation period, we could detect essentially no change in the free bilirubin or in the conjugated form. In addition, in following the patient's electrolytes over a three hour period, the CO_2 dropped from 20 mEq. to 8 mEq. At that point, we became anxious and administered sodium bicarbonate. Two replacement transfusions were then necessary to control the bilirubinemia. We would therefore like to add a further word of caution about the administration of glucuronic acid intravenously, and we question its effectiveness in most cases.

DR. LOUIS K. DIAMOND, Boston: I would like to make two points regarding Dr. Danoff's presentation. In the first place, anyone who has followed enough of these babies has undoubtedly seen a number of them suddenly begin to lose the indirect bilirubin after 48 or 72 hours. The slides that Dr. Danoff presented show that bilirubin values in some of these babies are already on the way down and falling rapidly after 72 hours. In such cases it makes use of the drug seem rather futile. In the second place, I think we have at least nine years of experience at Boston Lying-In Hospital without a single case of kernicterus in babies receiving exchange transfusions. To expose any infant, as Dr. Rudi Schmid has pointed out, to the danger of kernicterus when we have a successful treatment with a relative low incidence of complications, and to try this experimental treatment in the face of an effective one seems unjustified, particularly since the paper published in March suggests that three of the infants did develop kernicterus, possibly while receiving this treatment. Finally, I would like to ask Dr. Danoff what happens to the Coomb's positive cells in his patients, because it is widely held that these cells are destroyed eventually. If they are not removed, many of these infants do develop severe anemia about the third or fourth week. I wonder whether in the series treated by Dr. Danoff and Dr. Holt there was any evidence that these cells were protected in any way by this treatment. Did they also rapidly leave the circulation and did these infants require multiple transfusion?

DR. GERALD F. LUCEY, Burlington, Vt.: I would like to call attention to a study which will be published in *Pediatrics* in June of a series of patients who were treated at Babies Hospital, New York, a few years ago with sulfisoxazole (Gantrisin) therapy. Bilirubin levels were followed very closely. There was an equally "dramatic fall" in infants who developed kernicterus! This group of nine infants had proven kernicterus. The fall in bilirubin level can be shown to occur during the development of kernicterus or as the infant is dying. We hypothesize that what actually happened was that: sulfisoxazole acted in some way to change cellular permeability to bilirubin. A falling level of bilirubin may not always be a good sign.

DR. STUART DANOFF: I think I had better take the glucuronic acid myself. I do not see any point in answering each individual. We can group these criticisms into three or four main categories. First, Dr. Stullberg brought up a question as to the interference with the diazo reaction. We do not believe this is the case. We have added glucuronic acid in vitro and have not been able to interfere with the reaction. If it did interfere with the reaction, we would expect everybody to have marvelous results. The second point as to the possibility that we are driving bilirubin into the tissues rather than out of the body is, of course, a serious one, and one which has disturbed us a great deal. Dr. Lucey mentioned the experience at Babies Hospital where sulfisoxazole given to premature infants lowers the serum bilirubin and produces kernicterus. I would like to state once more that in all of our cases that have responded, we have seen no evidence of kernicterus. In the intravenously treated group, there has been no toxicity. This group was treated with sodium glucuronate, not glucuronic acid. Also, I am interested in Dr. Odell's observations on the different effect of sulfisoxazole and sodium glucuronate on bilirubin permeability. Dr. Day's Gunn strain of rats are rather disturbing. However, the difference between rats and babies is quite obvious, and these rats never can form glucuronides, or they only form a negligible amount, if any. Of course, a significant number of these rats develop kernicterus anyway. Another point is one which Dr. Day has not mentioned, but he told me about it. That is that the subcutaneous fat in the rats becomes yellower after sodium glucuronate, which is an exceptionally good indication that, in the rats, sodium glucuronate is pushing the bilirubin into the lipid substances of the body. He mentioned to me that he could see this difference in fat biopsies. This led us to do fat biopsies on some of our babies before and after sodium glucuronate administration. It was obvious that the fat was

not yellower. When we extracted the fat and bilirubin in chloroform and measured the amount of indirect bilirubin, the level in the B specimen was 50μg. per gram of fat. In the A specimen, the bilirubin concentration after glucuronic acid was 9μg. per gram of fat. We have done fat biopsies in two other babies who have shown an equally dramatic drop in serum bilirubin. In both cases, the bilirubin content of the fat decreased coincident with the drop in serum bilirubin. Therefore, we think this is another piece of evidence which indicates that the rats are very different from the babies and that the bilirubin is not being forced into the subcutaneous fat. As far as the discrepancy in the results is concerned, I think that the five cases treated with sodium glucuronate intravenously in Boston, except for the one case that I supervised, were not treated in the same was as our cases. Three of the babies received, not 200 mg/kg/hr., but 30 to 40 mg/kg/hr., not for 8 to 12 hours as we have recommended, but for 2 to 3 hours or 3 to 4 hours. Therefore, the three or four cases which did not respond are not comparable at all. The case that I visited Boston about had a bilirubin level of 12 mg. %, I believe. This baby did receive the proper dosage, but the bilirubin did not fall. I do not know how to explain this except that we have never given sodium glucuronate intravenously to a baby who had a bilirubin level this low without an incompatibility. We have only given it to babies who have incompatibilities in this range. We wait until they are about 15 mg. % if they do not have an incompatibility. I do not believe that the five cases treated with sodium glucuronate intravenously are comparable to our group. As far as Dr. Waters' case is concerned, I assumed he was using glucuronic acid and not sodium glucuronate, but I cannot explain his one case which did not respond. We have performed hematocrits on these babies, and there does not seem to be any indication that there is a dilution phenomenon. Some of these babies do become anemic, Dr. Diamond, and do require transfusions later on. The observations of Dr. Hsia are also very interesting. However, the adult dog does not lack the enzyme necessary for the production of bilirubin glucuronide. It is conceivable that the other pathways, if they exist, exist only in the newborn infant who does lack this enzyme. In summary, I still believe that we have demonstrated that this form of therapy is effective. I do not think that you should all go home completely discouraged. This does require an adequate trial.

DR. L. EMMETT HOLT JR.: Dr. Gellis, may I put in a word?

DR. GELLIS: You may, Dr. Holt, as long as you do not show a slide.

DR. HOLT: I shall not show a slide. I shall merely make the comment that Mollison and others have recently pointed out that exchange transfusions still cause a considerable number of deaths.

13. Congenital Malformations of the Heart in Siblings

ROBERT A. MILLER,* and EVANGELIA ZERVOPOULOS,*
Children's Memorial Hospital and Northwestern
Introduced by DAVID YI-YUNG HSIA.

Between 1952 and 1957 approximately 3000 sets of parents were referred by their physicians to The Children's Memorial Hospital because of a congenital malformation of the heart in one or more of their children. In 17 families, 2 siblings had congenital heart disease, and in 3 families, 3 siblings were affected. These 20 families and their 43 children with congenital heart disease constitute the material for this study.

After diagnostic study was done in these 43 cases, the malformations were classified embryologically. For example, lesions such as the transposition complexes, tetralogy of Fallot, or truncus arteriosus, were considered "early" that is, stemming from the time of rotation and involution of the bulbus cordis, whereas, lesions such as patent ductus, coarctation of the aorta, or atrial septal defects were considered "late," or stemming from the time of completion of septation, or later.

It was found that if one child had a complex "early" anomaly, his siblings with congenital heart disease also had a lesion in the same group. If one child in the family had a "later" anomaly, his affected siblings had a lesion which also was in the "later" group. In these 20 families, there was no mixing of "early" and "later" anomalies in the same family.

This pattern in the 20 groups of siblings studied, seemed more than coincidental and suggested a genetic factor in these families. The following studies were done:

1. A genetic history was obtained in the 20 affected families.

2. Similar histories were obtained from 20 sets of parents with one child with an "early" (transposition, etc.,) anomaly.

3. Similar histories were obtained from 20 sets of parents with one child with a "later" (ductus, coarctation, etc.) anomaly.

4. Similar histories were obtained from 20 sets of parents with no child with congenital heart disease.

This data will be shown in tables.

DR. ALEXANDER NADAS, Boston: I think this is a very interesting group of patients which Dr. Miller has presented. Obviously, however, they represent a minority; most of the children who have congenital heart disease do not have siblings

*By invitation

with congenital malformations of the heart. This is a very important point to stress because in our daily practice, dealing with these individuals, the parents always ask the question: how likely is it that I will have another child who will have a similar malformation? I think that if one can reassure them that it is very unlikely, it is important. Of course, it is much more likely that a second patient will occur in the same family than a first will, but I think, as physicians, we probably ought not to stress this, but simply say that it is quite unlikely. If one wants to quote figures, I think it is reasonably well accepted that the likelihood of a first child with congenital heart disease being born into a family is somewhere in the neighborhood of 8/1000. The possibility that the parents of this patient will have a second such child is somewhere in the neighborhood of 2 or 3/100. This is an increase, but I think the parents will feel better, and you are truthful, if you say that they have a 97 or 98% chance of having normal children. It is just expressing it in another fashion. May I show one slide, Dr. Gellis? Incidentally, Dr. Miller asked me to show this. This is our experience with familial incidence of congenital heart disease. We have a total of 17 families. Four families had more than two members affected. Now, I think I would like to jump to the bottom and simply say that of the 17 families, identical or similar malformations occurred in 11. This brings out the point that Dr. Miller was making, which is something that Dr. Paul Wood also said in England, namely, that if one patient has one type of congenital heart malformation, the second child in the same family with congenital heart disease will probably have the same thing.

Dr. George Husson, Syracuse, N. Y.: Dr. Miller, we have one rubella syndrome with a transposition complex. The history of rubella following pregnancy is well documented. In addition, we have one family with two female siblings. The eldest of these siblings had a mixed type with a transposition type complex in association with a large ventricular septal defect. She died following a brain abscess. The younger sibling has a patent ductus arteriosus.

Dr. Robert L. Brent, Philadelphia: I noticed in your abstract that you used the words "late" and "early." Yet, you omitted these words during your presentation. You are to be congratulated if you realized that ambiguity of these words in the field of teratology and how they can be misconstrued. When one says "late," does one mean the occurrence was late, the cause was late, or the last time in which the malformation could be corrected in utero was late. From a medico-legal standpoint, it can be an important word for a physician to use. Secondly, in analyzing this data, did you take into account the probability of congenital heart lesions occurring in 3000 families without a previous congenital heart lesion. For with an incidence of 5/1000, one would expect 15 congenital heart patients, if one child followed the original case-finding patient; 30 congenital hearts if there was an average of 2 children following the case-finding patient, and 45 congenital heart patients with an average of three children in these families minus the case-finding patient. Finally, I am not too sure of what you infer about the genetic causes here. I think you mean that the possible transposition time of malformations is more likely genetic in etiology. If this is true, of course, predetermination is at the time of conception.

Dr. Maurice Young, Vancouver, B. C.: We have been interested in this topic in Vancouver. Recently, we have been studying 40 families among 1200 cases. That gives an unusual incidence of about 3%. Our families included not only siblings, but also a father and daughter, cousins and an aunt and uncle, and one pair of twins. We have two instances of three congenital malformations in a given family and one instance of four malformations. So far, these cases have not been completely worked up, but the preliminary findings support the results given in this paper, namely, that in any given family, the so-called transposition group tends to recur again. We have had one case of a true truncus arteriosus, and we suspect a true truncus in another surviving sibling. It is perhaps of interest that of these cases, 25 of the families were encountered in the first five years, whereas we have encountered 15 in the past year. This suggests that, as usual, the more one looks for an abnormal condition, the more one finds it.

Dr. John M. Craig, Boston: I think there is one other group of conditions in which there have been recorded lesions in siblings. This is congenital endocardial fibroblastosis. One must admit there is argument about the true etiology of the lesion, but Dr. Paul Rosahn gathered from the literature six pairs of siblings with congenital endocardial sclerosis.

Dr. Robert A. Miller: I want to thank all of the people for their comments. In regard to what Dr. Nadas said about the questions parents may ask concerning subsequent children with congenital heart disease, I do want to point out that these 22 families were culled from between 3000 and 3500 families with congenital heart disease. We observed one sibling with transposition and a patent ductus in a rubella pregnancy. As I said before, there is no reason to think that these two groups are mutually exclusive no matter how we explain them. If, just for the purposes of speculation, we say that the transposition complexes may be more likely to be genetically conditioned and that the other types of congenital heart disease are more

likely to be influenced by other factors, a woman who has given birth to one child with a transposition complex can, in a subsequent pregnancy, by chance have a child with a lesion in the other group. Concerning the question of fibroblastosis, first of all, I think it should be mentioned, as long as we did bring up fibroblastosis, that there is probably more than one kind. One type of fibroblastosis which we see occurs with other congenital anomalies and may have an origin, partially, in hemodynamics. The other type of endocardial fibroblastosis which occurs in the absence of any other type of congenital heart disease stems from a different etiology. We have not seen the so-called primary type of endocardial fibroblastosis in siblings.

14. Morphological Changes in the Brain in Experimental Hypernatremia

LAURENCE FINBERG and CHARLES LUTTRELL,*
Johns Hopkins and Baltimore City Hospitals

Hypernatremic dehydration in infants has been reported to be accompanied by symptoms and signs of nervous system injury. Moreover, anatomical lesions have been observed in infants who died following this physiological disturbance. Studies on kittens with experimental hypernatremia previously reported have indicated changes in the "chemical anatomy" of the brain. In addition gross and microscopic morphological changes have been observed in the brains of 26 out of 30 kittens in which hypernatremic dehydration was induced by the intraperitoneal injection of a 1.5M sodium chloride—bicarbonate solution. Ten animals died before 24 hours had elapsed, the others were killed 24 hours after injection.

The gross findings were visible hemorrhages most often intradural, located chiefly over the occipital poles, the cerebellum and along the superior saggital sinus. These were one to six mm. in diameter. Large subdural hematomas and subarachnoid and intracerebral hemorrhage were also encountered. All of the animals with abnormal findings showed some accumulation of xanthochromic or sanguinous fluid over the brain.

Histological changes consisted of dilated veins and capillaries with hemorrhages particularly prominent in the sulci. These changes are quite similar to changes observed recently in a hypernatremic infant who died. The kitten brains also showed definite evidence of necrobiotic changes in the molecular or outer cell layer of the cortex indicated by edema, loss of staining reaction, and shrinkage of microglial nuclei.

Similar gross and histological changes have also been produced in animals injected with hypertonic (3M) urea solutions, although the clinical behavior and "chemical anatomy" of the tissues of the latter animals differ from those of the sodium loaded animals. Hence the changes may not be specific, but they are strikingly consistent in occurrence in these animals and they resemble what has been seen in infants. The mechanism of hemorrhage is not completely clear but our studies suggest that it is preceded by vascular engorgement. It seems likely that at least part of the permanent residual nervous system damage observed in infants following hypernatremic dehydration is related to changes of the type observed here.

Discussion

DR. WILLIAM B. WEIL JR., Cleveland: It has been our impression that in comparable degrees of hypernatremia, children whose elevation of sodium and chloride has resulted from the administration of salt orally showed less severe symptomology than those children who had not received oral sodium chloride. If this is true, it would suggest that there was more than simple elevation of serum sodium as the etiologic factor in the production of the signs and symptoms in this state. The other point I would like to make is that the children who develop this complex develop it rather slowly and the tremendous shifts of fluid which would occur as the result of the intraperitoneal administration of three molar sodium chloride would not seem to occur to this degree in the children. I wonder if Dr. Finberg would care to comment on that.

DR. CLEMENT A. SMITH, Boston: Dr. Weil asked, in a way, the question I was going to ask which comes from having so often seen newborn infants, given no water for supposedly therapeutic purposes, develop quite high serum sodiums—160, 165 sometimes. I am wondering whether you have produced hypernatremia by giving newborn kittens no water, so as to concentrate serum sodium, to see whether this has the same effect as the changes you found in the brain.

DR. ROBERT W. WINTERS, Chapel Hill, N. C.: I would like to ask Dr. Finberg if he measured blood pH and calculated P^{co_2} in these animals. I mention this because Winkler, Elkinton, and colleagues, I believe, made the point that hypernatremic animals die with what they thought was central respiratory depression. If it turned out that P^{co_2} were elevated under these circumstances, this might add an important factor to the pathogenesis of the lesions which you have observed.

DR. RANDOLPH K. BYERS, Boston: We have a small number of children who have been found to have elevated serum sodium to a considerable degree—160, 170 mEq., something of that sort—who have grown up and are having a great deal of trouble with a type of learning difficulty. They have difficulty with attention and form perception,

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things which are associated with brain injury. It seems to me that the three I am thinking of were all infants who were overtreated with intravenous sodium chloride solutions. They are now six or seven years old, and they all have this brain injury syndrome, which is quite striking and very difficult to manage.

Dr. Donald B. Cheek, Dallas, Texas: I would like to congratulate Dr. Finberg on his paper. I have one question to ask. Recently, we observed in three or four of these children a low urine concentration and a fall in the urine specific gravity (1.003 to 1.005). At the same time, we have seen patients with this syndrome who have concentrated the urine satisfactorily. These are circumstances where one would expect that the concentration of urine would be a maximum, and I wonder whether Dr. Finberg could throw any light on these findings or whether he may feel that some coincidental disturbances of antidiuretic hormone might initiate this whole business.

Dr. Nathan B. Talbot, Boston: In keeping with this problem, from a therapeutic point of view, I am wondering if you would work out some way to repair this form of dehydration in a manner which is likely to avoid the occurrence of convulsions which are so often seen on the first or second day of therapy.

Dr. Laurence Finberg: A great many provocative and stimulating questions have been asked. I will try my best to answer them. Dr. Weil made two points: one was that, in his experience, the infants who had ingested salt seemed to have less symptomatology, if I understood him correctly, than those who acquired water deficits. Our experience does not quite coincide with that, although I will readily agree that the extent of symptoms does not correlate well with a particular sodium level. Some of our most symptomatic and permanently brain-damaged infants were those who received large amounts of salt. One of these was an infant fed salt inadvertently by his mother, who had substituted it for sugar in the formula. He became hopelessly vegetative subsequently, although he survived. Dr. Weil's comments about sudden change, I am sure, are valid, and it is a limitation in carrying over the experimental animal results to human experience. However, there are so many similarities that it seemed worth while to do these experiments and report the results. Dr. Holliday, thank you for your additional evidence. I think that Elkington and Danowski also produced similar evidence about 15 years ago in a paper which was published in The Journal of Clinical Investigation. Dr. Smith, in 24 hours' time, with the kitten, you do not get sodium concentrations as high as this. If you wait four days, then it is hard to make a comparable experiment. I cannot really answer that question. Dr. Winters, we unfortunately did not measure pH and P^{CO_2} in these animals. I think your comment is correct. Clinically, the animals we were studying did not seem to have anything resembling a central respiratory depression. I might comment that CO_2 content was not outside physiological limits. Dr. Byers, we have observed the same sort of thing in a number of children. Dr. Cheek, we have made the same observation concerning infants that you have, that there are some who, under these conditions, put out dilute urines despite hypernatremia. The kittens under these conditions did put out maximally concentrated urines. Dr. Talbot gets to the most important clinical question, of course. Our experiments do not bear on that directly. I do not have an answer as to what is the best way to repair the dehydration of these infants, except to say that I would think that slowness of therapy would be the most important point. There is no hurry to get the serum sodium concentration down to normal. If this type of intracellular osmotic change is going on in humans as well as in animals, any rapid shift might be expected to be associated with the change in function whether going in the direction of repair or in the direction of injury. This is the same thing, I think, that we have talked about as resembling water intoxication when you give water to these patients too rapidly.

15. An Evaluation of T_{m_c} H_2O in Children with Sickle-Cell Anemia

Charles F. Whitten,* and Antoine A. Younes,*
Receiving Hospital, and Wayne
Introduced by Wolf W. Zuelzer

It has been established that individuals with sickle-cell anemia have a defect in renal concentrating ability when evaluated by measuring the maximal urinary osmotic concentration attained during antidiuresis. The purpose of this study is to appraise the concentrating defect in children with sickle-cell anemia by utilizing a second index of concentrating ability, namely the maximal rate of solute-free water reabsorption achieved osmotic diuresis (T_{m_c} H_2O).

After a 13-15 hour period of total deprivation of water and food, osmotic diuresis was achieved through the intravenous infusion of 10% mannitol at a rate of 20 ml/min/1.73 square meter. The rate of solute-free water reabsorption (T^c H_2O) was calculated from the following formula:

$$T_c\ H_2O = \frac{U_{osm}\ V}{P_{osm}} - V, \text{ or } C_{osm} - V$$

where

U_{osm}=urine osmotic concentration

V=urine flow in ml/min.

P_{osm}=plasma osmotic concentration

The maximum rate of solute-free water reabsorption (T_{m_c} H_2O) was then derived as the mean of

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all values of T^c H_2O falling within the rectilinear portion of the line of regression obtained by plotting C_{osm} against V. Other observations include maximal urine osmolarity, maximal osmotic U/P ratio, GFR, ERPF, rate of urine flow during hydropenia and the pre and post levels of plasma sodium and potassium.

In 16 children with sickle-cell anemia the mean T_{m_c} H_2O was 4.5±0.9 ml/min/1.73 square meters (range 3.2-6.2) as compared with a mean of 5.2±1.4 ml/min/1.73 square meters (range 3.4-7.5 in 12 normal children. The other pertinent results are presented in the accompanying table.

	Max. Urine Osmolarity	Max. Osmotic U/P Ratio	T_{m_c} H_2O Max. Os. U/P Ratio
Normal			
12 children	Range 807-1210 mosm/L	2.8—4.4	0.8—2.1
(15 mo.-14yr.)	Mean 1056±128 mosm/L	3.8±0.5	1.4±0.4
SCA			
16 children	Range 429-664 mosm/L	1.6—2.4	1.7×3.4
(22 mo.-13yr.)	Mean 542±68 mosm/L	2.0±0.3	2.4±0.4

Our results indicate that the effect of sickle-cell anemia T_{m_c} H_2O is minimal in comparison to its effect on maximal urine osmolarity and maximal osmotic U/P ratio. This might be restated to say that when the amount of osmotic work to be accomplished is small (as exists during osmotic diuresis) the renal tubules in patients with sickle-cell anemia are able to reabsorb a basically normal amount of solute-free water. However, when the osmotic gradient is large (as exists during hydropenia) the ability to reabsorb solute-free water is impaired. Whether this can be attributed to an aberration in renal tubular energetics or is a result of an alteration in renal tubular membrane permeability is unresolved.

This is the first disease state described in which it has been demonstrated that T_{m_c} H_2O has a substantially normal value while the maximal urine osmotic concentration attained during hydropenia is definitely decreased. The fact that this dissociation can occur would suggest that in terms of normal renal physiology these two indices of water reabsorptive ability have different functional significance.

Discussion

Dr. Philip L. Calcagno, Buffalo: This is a situation in which there is a difference in the mechanisms of renal concentrating capacity. Certainly, the concentrating mechanism defect at low urine flows has been described years ago. Now, to find that a normal concentrating mechanism at high urine flows exists in sickle-cell anemia is a situation which is most interesting. We have seen functions of the kidney which apparently are related to the same anatomical site become separated by disease or growth. For example, the premature baby, with regards to the diluting mechanism, matures quickly in life, while the concentrating mechanism matures at a slower rate. I should like to ask Dr. Whitten whether or not he has done any studies with sickle-cell trait patients? I think this might prove interesting. Secondly, I wonder whether or not he has any data regarding glomerular filtration rates. We have seen patients with supernormal rates, normal rates, and low glomerular rates. I should like to know how many patients with sickle-cell anemia had low glomerular filtration rates.

Dr. Malcolm A. Holliday, Pittsburgh: I find it difficult to understand how T_{m_c} H_2O can be normal at a time when urinary concentrating ability is impaired. The mechanism and site of absorption is the same in contrast to mechanisms resulting in dilution of urine. I would suggest that the hydration attending the measurement of T_{m_c} H_2O may restore the mechanism for water extraction to normal whereas the circumstances attending the concentration test may result in its impairment. In other words, the two observations were not measured under comparable conditions. Do you have any information on histologic appearance of renal tubules from children with sickle-cell anemia. I wonder if you have any data on their ability to concentrate under conditions of increased oxygen tension or in response to vasopressin (pitressin) without dehydration.

Dr. Robert E. Cooke, Baltimore: I would like to ask whether or not the capacity to concentrate has been tested with urea loading? This question is asked particularly in regard to the demonstration by Epstein and others that maximal capacity to concentrate is markedly increased by urea loading, in contrast with other solutes.

Dr. Robert W. Winters, Chapel Hill, N. C.: I would like to know whether or not you have employed maximal dilution with the patients that you described in this report, and particularly, what was the least osmotic U/P ratio that could be achieved with maximal water load? I wonder if this could give you any kind of insight into the magnitude of the gradient which could be established in these patients; perhaps, if differences from normal were found, it might suggest that the gradient is not as great in these patients as it is in normals.

Dr. Jack Metcoff, Chicago: I wonder if you have had an opportunity to administer transfusions to any patients with sickle-cell anemia and to determine whether there had been any alteration in T_{m_c} H_2O under such conditions?

Dr. Wallace W. McCrory, Philadelphia: I too am quite interested in this paper. I think this

attempt to measure the T_{m_c} H_2O in such patients is laudable. The finding that there is no statistical significance in the differences found in these two groups does not necessarily imply that there is no physiologic meaning to differences that may exist. The glomerular filtration rate, renal plasma flow, and maximal capacity for PAH excretion are definitely supernormal in children with sickle-cell anemia. It is thus of interest that the authors fail to find a similar difference in this renal function in these patients. It is also important to remember that T_{m_c} H_2O is not a constant. It can be changed by varying the protein content of the diet and possibly other factors. I would think that some of these other factors need to be considered before we can completely resolve the questions raised by the work presented by these authors. I would like to ask a further question concerning a different explanation for the abnormality in renal tubular function seen in this disorder. The current hypothesis concerning the mechanisms for renal water absorption and urine concentration were discussed briefly this morning by Dr. Brodsky. A vascular counter-current flow system in the medulla and papillae of the kidney is considered an integral part of this mechanism. The efficiency of the concentrating mechanism will then depend fairly directly on the integrity of the blood vessels making up the counter-current flow scheme. I would wonder then if patients with sickle-cell anemia may not be very susceptible to interference in the integrity and organization of such a vascular counter-current system in the renal papillae. During periods of sickle-cell crises, occlusion of smaller vessels could occur particularly in this vulnerable area of the kidney. Any disorganization in vascular supply in this region could be associated with some loss of efficiency of the concentrating mechanism. Such a process would produce qualitative changes varying with the degree of anemia and the duration of the disturbance. If this is to be demonstrated, one may then find only small degrees of differences in this function in children but these differences will become much greater the longer the duration of sickle-cell anemia. I would be interested in the authors comments on this hypothesis.

DR. ROBERT KLEIN, Pittsburgh: In support of what Dr. McCrory said I wonder whether the mere increase in hydration from the loading that occurred may not have effected this change in the same manner that transfusion or water-loading intravenously ameliorates the crisis of sickle-cell disease.

DR. WILLIAM B. WEIL JR., Cleveland: I would like to ask whether there was a difference between the two groups in regard to the rate of urine flow at which the T_{m_c} for water was reached.

DR. CHARLES F. WHITTEN: I certainly want to thank the many discussers, and I will try to answer as many questions as possible. Dr. Weil, we do have a very similar range of critical values of urine flow at which the T_{m_c} water first reaches a value you can measure. The mean of 6.6 ml. per minute in the normal group and the mean of 7.7 ml. per minute in the sickle-cell anemia group is relatively comparable. Dr. McCrory, I think the point we would like to stress is that there is a difference in the maximum urinary osmotic concentration and the T_{m_c} water. Now, this is irrespective of mechanism. I do not think the theories of renal concentrating ability extant at the moment would permit, on this type of data, a firm statement as to actual mechanism. The data, I think, is fairly clear in showing that there is a difference between the two groups, irrespective of mechanism. Dr. Metcoff, we have given transfusions to five children with sickle-cell anemia, and I might say that we were able to confirm the observation that with long transfusions one can reverse the concentrating effect in the young infant. In two of the five, both of them infants, we were able to reestablish a normal level of maximum urine osmotic concentration obtained during hydropenia. However, one of the problems with measuring the T_{m_c} water is the question of reproducibility. In repeating our levels on three normal children and two children with sickle-cell anemia, we got variations ranging up to 1 cc. per minute. Now in the two individuals who were able to reverse the concentration defect through transfusions, we found that on repeating the T_{m_c} water, we got changes of 0.5 and 1.5 ml. Whether this 1.5 ml. represents entirely the effect of transfusion or is a part of the range of variation to be expected, we are not able to say at the moment. We think our data at the moment is too limited to present a firm picture on this problem. In the other three children, all over five years of age, we were unable to reverse the concentrating defect. Dr. Winters, from the standpoint of maximum water loading, I think there you are referring, not to T_{m_c} water, but to free water clearance. Dr. Cooke, I have no data on urea loading. From the standpoint of oxygen tension, Dr. Holliday, in the work of Dr. Keitel, when individuals were subjected to increased oxygen concentrations, they maintained normal oxygen tensions or elevated oxygen tensions. This did not influence maximum urine osmotic concentration. Dr. Holliday, with respect to histologic appearance, we have reviewed the slides of eight children who had sickle-cell anemia and who died at Children's Hospital in Michigan. We were not able to find a lesion that we could correlate with the functional studies. Dr. Calcagno, with respect to glomerular filtration, we find a wide range in children with sickle-cell anemia. It varies from 70 ml. per minute to 220 ml. per minute. There is no correlation between glomerular filtration and the T_{m_c} water or

maximum urinary concentration. Finally, with respect to sickle-cell trait, we performed a simple concentration test on some 50 children with sickle-cell trait, with 30 normal children as controls. We have found that only 12 of this number were unable to obtain a maximum urine osmotic concentration above 800 mOsm. per liter. We concentrated particularly on children below five years of age. This is in contrast to the data reported in adults. It has been reported that 88% of some 34 adults with sickle-cell anemia were unable to achieve this level of concentration. Others have reported that 70% of 20 adults were also unable to concentrate to 800 mOsm. per liter. We feel that this great difference in the incidence of the concentrating defect in these two age groups, coupled with the fact that the lesion can be reversed in young infants with sickle-cell anemia, gives evidence that the concentrating defect in association with sickling phenomena is an acquired progressive lesion rather than one which is genetically determined.

16. Human Growth Hormone in Human Biologic Fluids

D. K. Keele,* H. Mansmann,* and R. Klein, Pittsburgh

An attempt has been made to develop a clinically feasible method of assaying growth hormone in human biologic fluids. Antibodies against bovine growth hormone have been produced in the rabbit by the injection of the growth hormone material in Freund's adjuvant. The antiserum thus produced was capable of precipitating growth hormone completely out of solution. That is, when such a precipitation was carried out and the supernatant fluid was injected into hypophysectomized rats, there was no growth activity. Growth hormone mixed with normal rabbit serum was as active as the same amount of growth hormone by itself. When the precipitate formed from the union of growth hormone and the antiserum was injected into hypophysectomized rats, there was a varying growth response. In two instances significant, growth was produced and in the other three instances, there was no significant growth as measured by the width of the tibial epiphyseal cartilage.

The antiserum was then tested against various beef and human protein products in the gel-agar system described by Oudin. Cohn fraction II-III was the only human serum fraction precipitated by the antigrowth hormone serum. We did not separate this fraction cleanly enough, and both the fraction II and the fraction III that we prepared gave positive reactions. However, it is our feeling that the antigen will properly be found in fraction III.

A marked precipitin reaction was formed by the antiserum and serial dilutions of this fraction made in our laboratory from the individual sera of children and adults. Fraction III-2 obtained commercially also gave positive results as did a single untreated diabetic serum and a β lipoprotein fraction obtained by continuous flow electrophoresis. No reactions were produced with normal human serum, human albumin, fibrinogen, γ-globulin, fraction IV-V, and fraction II-III prepared from cord blood serum and from a 14-year-old girl who was dwarfed following removal of a craniopharyngioma. Bovine serum albumin, insulin, thyroid stimulating hormone, HOG ACTH, and pitressin were also not precipitated, though beef fractions III-0 and IV were precipitated. Paper electrophoresis showed the growth hormone occupied the same area on the paper that fraction II-III occupied, and this was also equivalent to the rate of migration of the β lipoprotein obtained from the continuous flow electrophoresis. The combination of antiserum with fraction II-III completely precipitated out all the antibodies to growth hormone, and conversely the precipitation of growth hormone by the antiserum precipitated out all the antibodies against fractions II and III.

Neutralizing antibodies to bovine growth hormone have been produced in rabbits. The antiserum does not react with other pituitary hormones tested. Human serum β lipoprotein fractions have an antigenic material that reacts with antiserum in a fashion identical to that of bovine growth hormone. Antibodies against growth hormone can be adsorbed out by fraction II-III, and vice versa. Using this precipitation reaction we have begun to measure growth hormone in human serum. It is more plentiful in the serum of children than in the serum of adults or of infants. None was found in the serum of hypophysectomized 14-year-old girl.

Discussion

Dr. Charles H. Read, Iowa City: This paper and one which Dr. Hayashida presented recently in Buffalo at the meeting of the American Association of Anatomists have recorded the fact that an antibody can be produced to bovine pituitary growth hormone. We have prepared in rabbits an antibody to human pituitary growth hormone (Raben). The presence of this antibody has been demonstrated both by the Boyden technique and by the more orthodox complement-fixation technique. The human pituitary growth hormone antibody does not react with bovine pituitary growth hormone, pork ACTH, beef TSH or insulin. However, it does react with both Dr. Raben's and Dr. Li's preparations of human pituitary growth hormone and with Dr. Raben's monkey pituitary growth hormone.

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By using the hemagglutination-inhibition procedure we have been able to measure minute amounts of human growth hormone in serum. The data obtained so far suggests that in normal children and adults the levels are in the range between 0.16 and 0.64 μg. per milliliter of serum.

DR. MELVIN GRUMBACH, New York: I would like to congratulate Dr. Keele and his associates for this interesting and provocative paper. Perhaps we can look forward to an immunochemical method with a high degree of specificity for the detection of circulating growth hormone in man. Through the generosity of Dr. M. Raben, we have been able to obtain a small supply of human growth hormone. We have initiated studies to determine its effect on the growth of premature infants. Dr. Raben and others have shown that growth of hypopituitary dwarfs can be produced and maintained with as little as 6 mg. per week. On the other hand, the premature infant is relatively unresponsive to the administration of as much as 10 mg. of human growth hormone per day for three days. I would like to ask Dr. Keele whether he has had the opportunity to measure the concentration of growth hormone in the serum of premature infants or in cord and maternal blood? Assays of the growth hormone content of human pituitary glands at various ages have shown a relatively constant content of this hormone in relation to the weight of the gland.

DR. D. K. KEELE: Dr. Read is to be congratulated on his studies. That he has produced antibodies to human growth hormone is important. We have not tired to produce antibodies to human growth hormone as our preparation was inactive. In answer to Dr. Grumbach, I do not know why premature babies do not grow when given human growth hormone. I suppose that it is associated with end-organ responsiveness. The explanation may be that in very young infants the major stimulus to growth may not be growth hormone. For example, patients with hypopituitarism seem to grow moderately well in the first year or so of life. Slide 7 shows some preliminary studies made in the gel-agar system. We attempted to quantitate the concentration of growth hormone in human fraction II and III by measuring distance of migration of the zone of precipitation. No reaction occurred using fraction II and III from a 2-year-old. This method shows greatest reaction in age group 6 to 13. Fraction II and III from a hypophysectomized patient gave no reaction. The problem now is to prove that what Dr. Read is measuring and what we are measuring is really active growth hormone.

17. Factors Affecting Fluorescence of Antibody-Labelled Erythrocytes

JOANNE WHITAKER*; WOLF W. ZUELZER; ABNER F. ROBINSON,* and MARGARET M. EVANS,* Wayne

The demonstration of antigen-antibody reactions involving erythrocytes by the fluorescent antibody technique has been unsuccessful to date, and to our knowledge no reports on this subject have appeared in the literature despite its obvious theoretical and potentially practical significance. A variety of specific antigen-antibody reactions involving tissues and microorganisms, on the other hand, have been readily demonstrated by the fluorescent antibody technique. This study was undertaken to investigate and possibly overcome factors which might be responsible for the special behavior of erythrocytes in this respect.

The assumption was made that the failure of antibody coated erythrocytes to fluoresce is due to a "quenching effect" related to structural or metabolic factors in the red blood cells rather than to insufficient number of acceptor sites. Attempts were made to elucidate this apparent "quenching effect."

In preliminary studies, it was shown that fluorescent dye-labelled antiserum actually attaches to the red cells, since cells coated in this manner gave a positive Coomb's test. It was possible, moreover, to elute the labelled antibody from the red blood cell and again demonstrate fluorescence in the eluate. This indicated that neither the linkage of the antibody to the antigen, nor the bond between the labelling dye and the antibody protein were being affected. A true but temporary quenching effect was thus demonstrated.

To study the nature of this phenomenon, the effects of various oxidizing and reducing agents were investigated, and it was found that all agents destroying SH group linkages were effective in producing quenching of the fluorescence. Since it is known that heavy metals quench resonance, a number of different chelating agents such as versene, BAL, and 8-oxyquinoline were investigated next. It was possible by pretreatment of the erythrocytes with these agents to abolish the quenching effect and to demonstrate fluorescence in such systems as labelled anti-A versus group A cells. The labelled erythrocytes gave a moderate amount of specific fluorescence which will be demonstrated. When already sensitized, Coomb's positive erythrocytes were treated with versene and it was observed that agglutination in the Coomb's test was significantly weakened or abolished. The implication of these findings will be discussed.

* By Invitation

Discussion

DR. KURT LANGE, New York: I wonder whether Dr. Whitaker would be willing to accept a somewhat simplier explanation of the phenomena that she has found. We have been interested in the fluorescence of tissue after injection of fluorescein. A constant observation in these studies is that over an area where there are pooled red cells, the intravenously injected fluorescein does not give the same degree of fluorescence, but a much lower degree. On examination of such an area and the obtaining of a few drops of the fluid, it can be shown that the fluorescein content is exactly the same as before. We are thus dealing with the filtering effect of the hemoglobin on the ultraviolet light. I wonder if the phenomenon is not a physical one and whether the versene reaction, that I would especially like Dr. Whitaker to tell us about, does not change the hemoglobin in such a way that it now becomes transparent to a certain extent to the ultraviolet light. In support of this is the observation that the red cell has a well-defined fluorescent ring on the outside where the layer of hemoglobin is thin in contrast to the center.

DR. CHARLES REINER, Brooklyn: We have been interested in this, and we have met with the same problem of quenching. We had some preliminary observations which seemed to indicate that perhaps hemoglobin may be an interfering substance when washed red cells are smeared and fixed in various fixatives. There is a difference in the staining capacity of these cells. The cells fixed in formal alcohol sodium acetate give a much more intense reaction than those fixed in the unneutralized formal alcohol. This may be a pH effect because we find that the formal alcohol sodium acetate fixative solution has hemoglobin pigment in it. We have been unable to obtain a high degree of specificity of reaction with our sera. We get almost as good a reaction with O cells as with A cells. We have not been able to explain this as yet.

DR. JOANNE WHITAKER: Dr. Lange, we were interested in the hemoglobin having a diluting effect on the fluorescence of the red blood cells also. In an attempt to decide whether the hemoglobin was causing the quenching effect of the red blood cells, we took some chemicals at random off the shelf. We took the fluorescent-labeled antiserum and observed it under Wood's lamp. When hemoglobin was added to the fluorescent material, the fluorescence was slightly diluted but the fluorescence was not completely quenched as it was when ascorbic acid was added. We think that the versene definitely does do something to the hemoglobin because the unversenized cells are bright red, whereas the versenized cells have an extremely dark red appearance which probably represents reduced hemoglobin. In observing the red blood cells the fluorescence of the versenized cells is usually more intense on the outside border of a large clump. In the middle of the clump where there is more hemoglobin the fluorescence is less intense. As far as the fixing agents are concerned, we think that the pH does have a great deal to do with the intensity of the fluoresence. Our previous difficulty had been in observing the fluorescence of red blood cells at all until after the cells were versenized. There was no difficulty in distinguishing the difference between O and A red blood cells after the labeled antiserum had been absorbed with mouse liver powder and red blood cell stroma.

18. The Electronic Control of Extra Corporeal Circuits by Muscle Action Potentials

RANDOLPH BATSON*; SAM E. STEPHENSON, JR.*; S. L. CLARK,* and L. H. MONTGOMERY,* Vanderbilt
Introduced by AMOS CHRISTIE.

This report concerns the development of an electronic device which is activated by impulses received from muscles undergoing contraction and permits control over motors, air valves, and other triggering devices. Electrical potentials, detected by implantation or skin electrodes, are passed through a tuned amplifier to exclude electrocardiographic and other undesirable potentials. The intensity of the isolated muscle action impulse is then increased to a value suitable for the operation of extracorporeal circuits.

Initial investigations were stimulated by the need for a device whereby patients with partial respiratory paralysis might operate respirators according to biochemical needs. Customarily the mechanical control of artificial respiration has been dependent upon machines regulated by an operator who makes adjustments to supply room air or other gas mixtures at a previously determined rate and volume. As a result, prolonged artificial respiration without frequent measurements of arterial blood values may result in states of hyperventilation or hypoventilation.

For three years the unit has been used in experiments on monkeys, cats, and dogs; in states of decerebration, with diaphragm paralyzed, with the thorax open, during pneumonectomy, and with the spinal cord severed at level C-2, obliterating all primary respiratory muscle function. In the latter case, dogs operate the device and control respiration by contraction of the small dilator muscle of the ala nasi. These animals rapidly "learn" that contraction of this small muscle is necessary for machine operation and that relaxation is necessary in order to produce passive expiration and in turn the initiation of another inspiration. Experiments

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on more than 80 dogs have proven the efficacy of the device in permitting animals to control their own respirations through physiological mechanisms. Animals used in these experiments made readjustments and quickly regained initial blood values after the experimenter had by interference brought about states of hyperventilation and hypoventilation. The control of respiration over periods of days in anesthetized animals has been achieved by impulses from needle electrodes in the ala nasi, the platysma, the intercostals and the diaphragm.

The unit has been used by several patients with partial respiratory paralysis. These patients have relied upon self-operated respirators for periods as long as several days regulating their own inspiratory rate and volume at will. In these instances, impulses were received through skin electrodes placed over primary or accessory muscles of respiration. During operation adjustment of the electrodes or the controls of the electronic unit is unnecessary and patients sleep normally. Considerable satisfaction seems to be enjoyed by the patients who are able to control respiration and produce an occasional long deep breath.

A modified device is available for use by patients during open-chest operations. It is arranged in such a fashion that the rate and depth of inspiration is controlled through a positive pressure machine and endotracheal catheter, the machine being activated by muscle action potentials detected through needle electrodes in the patient's diaphragm. Measurements of alveolar CO_2 arterial blood pressure, EKG, and EEG will be simultaneously recorded during these procedures. The results will be reported.

The basic principles involved in the design of the equipment has been carried over to the development of transistorized models are to be used with portable equipment and in situations such as operating rooms where the sparking contact of the relays in the tube-type device would be hazardous. Attempts are now being made to modify the control circuit so that air and motor operated prosthetic extremities can be controlled by action potentials in neighboring muscles.

This device will be a valuable tool for investigations dealing with neuromuscular function, pulmonary physiology and the control of other extracorporeal circuits.

19. Coxsackie Infection in Infants and Children: (1) Clinical Aspects; (2) Pathology

D. M. Hosier * and W. A. Newton Jr.,*
Ohio State
Introduced by Warren E. Wheeler.

Coxsackie viruses are considered to be among the most prevalent of recognized viruses. They are known to cause herpangina, epidemic pleurodynia, and aseptic meningitis, but until recently have not been thought to cause death. To date, five reports have described fatal myocarditis in newborn infants caused by type-B-3 or B-4 Coxsackie virus. Four of these reports originated abroad in the Netherlands or in South Africa and described epidemics of fatal myocarditis occurring in newborn infants during epidemics of pleurodynia and/or aseptic meningitis in the population at large.

We are reporting the isolation of type-B-4 Coxsackie virus at autopsy from the myocardium, liver, brain, and kidney of a 14-day-old infant, dying of sepsis of unknown cause, and from the myocardium of an eight and one-half-week-old child, who died in heart failure.

Type-B-4 Coxsackie virus was isolated from the stool and a rise of homologous serum-neutralizing antibodies was observed in a 5-year-old boy, who developed severe myocarditis two weeks after a flu-like syndrome. He has recovered completely.

From this evidence, it appears that type-B-4 Coxsackie virus can cause death in early life, either by a localized infection of the myocardium, or by a generalized process involving myocardium, central nervous system, liver, and adrenal gland. The recovery of the virus in the stool coupled with a rising titer of homologous neutralizing antibodies strongly suggests that type-B-4 Coxsackie virus may cause nonfatal myocarditis.

We have reviewed our autopsy case material of infants and children showing focal or diffuse myocarditis, and a striking seasonal incidence was found. Seven out of ten of these children died during July, August and early September. This period approximates the peak seasonal incidence of Coxsackie virus infections. Five out of the ten children showed either focal hepatic necrosis or encephalomyelitis, or both, in addition to the myocarditis. This extracardiac involvement follows the distribution of lesions observed in our newborn infant whose tissues yielded type-B-4 Coxsackie virus. The seasonal incidence and the distribution of the extracardiac lesions suggest that Coxsackie viruses may be the etiologic agent of some cases of idiopathic myocarditis.

These cases are reported to stimulate further interest in studying living and dead patients with myocarditis of all ages and infants dying of unexplained sepsis. It appears that Coxsackie viruses may prove to be an important pathogen in these syndromes.

Discussion

Dr. J. D. Hansen, Capetown, South Africa: I would just like to mention that we in South Africa are becoming more and more aware of the danger of this Coxsackie myocarditis in the newborn. In the last six months, during the South African

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summer, we had two separate outbreaks in maternity homes. The first outbreak, I think, had about 10 infants infected with 4 deaths. The virus unit of the University of Capetown was able to carry out full epidemological studies and traced the infection to visitors who had come to see the mother in the maternity home. The second outbreak of infection was traced to a nurse who had the Coxsackie virus and who had complained of pleurodynia-like symptoms.

Dr. Leo J. Geppert, Washington, D. C.: This disease is not especially rare. With a little experience it can be suspected clinically. We have seen a number of cases including twins who developed the Wolf-Parkinson-White syndrome with their myocarditis. Two infants actually died from C. N. S. involvement rather than from myocarditis. I would like to make a plea for putting material in the deep freeze at postmortem on all children dying from possible viral diseases. After the pathologist has seen the sections, they can be discarded if the microscopic appearance does not suggest a viral etiology.

20. Pulmonary Ventilation in Infants Less Than One Year of Age

T. K. Oliver Jr.*; R. S. Shaw,* and W. E. Wheeler, Ohio State

Mechanical success in spirometry among infants requires that the spirometric apparatus have little or no resistance to air flow, no appreciable dead space, and a recording apparatus that turns at speeds to permit careful and precise dissection of the component parts of the respiratory cycle. A spirometer satisfying these requirements has been constructed and utilized in the measurement of pulmonary ventilation in infants ranging in age from 3 days to 8.9 months. The spirometer consists of a 27 liter air-tight lucite box in which is placed a 9 liter neoprene (weather) balloon. Both bag and box are connected via one-way valves to a minimum-dead-space infant face mask. A bellows of plastic film communicates with the box such that displacement of air from the box (inspiration) or air into the bag (expiration) causes movement of the bellows, to which is attached a constantin wire resistance strain gauge, connected in turn to a strain gauge amplifier and recorder. The response of the bellows-strain gauge system is essentially linear to 200 ml., and factitious bellows movement does not occur with varying respiratory flow rates. The recorder is run at 25 mm. per second.

It is possible to measure tidal volume (Vt), inspiratory time, expiratory time, total cycle time (T), respiratory rate (f), and flow rate during inspiration and expiration. Minute volume (V) can be calculated and alveolar ventilation (Va) can be estimated by assuming the dead space (Vd).

Infants were studied after meals, and they were either asleep or very drowsy and were believed to be in a steady state. In all of the infants it was possible to obtain a tracing of greater than 60 seconds duration and repetitive tracings have been possible in most. The irregularity in respiratory rate characteristic of neonates has not been observed in infants over one month of age.

Statistical analysis of prolonged tracings or repetitive tracings indicates there is no trend in Vt or f, from which we conclude there is no build up of CO_2 within the face mask or evidence of altered ventilation due to pressure of the face mask.

Data on 49 infants, about half the number on which measurements were attempted, are satisfactory and are reported here. The results have been compared with age, weight, and body surface area. The latter gives the best correlation in the range studied (0.16-0.40 square meters). There is a progressive increase of Vt, V, and flow rates with age, weight, and body surface area and a decrease in f.

Also shown are breathing patterns in infants with laryngeal stridor and bronchiolitis.

Discussion

Dr. Clement A. Smith, Boston: This is the kind of report in which one would rather see the machine in action than the data. The data looked very good and very useful. Your record of getting every other infant to sleep during your measurements shows that you have done very well indeed. I see that Dr. Cook is on his feet, and he may have some much more technical question to ask, but I do know what a lot of work goes into getting this kind of data. I congratulate the authors on having gotten so much.

Dr. Charles D. Cook, Boston: These measurements of respiration in the age group between the newborn infant and the cooperative child of five or six years of age are interesting and important, as very few studies are available. This technique appears to be one which can be usefully applied to a number of conditions. I have several technical questions: what is the dead space or volume of the mask? Since the dead space of a newborn infant is around 5 or 10 cc., a very small increase in the dead space of the apparatus would make an appreciable difference in the child's ventilation. I would also like to question the use of surface area as the basis of comparison of the various measurements, as it involves many assumptions. Finally, has this technique been used for older children of a few years of age?

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DR. ALFRED H. WASHBURN, Denver: I really did not intend getting up, but seeing my name at the top of that slide stimulated me to get up and make a coment or two. I think perhaps one reason for the difference between the volume and rate figures in the babies that Dr. Deming and I studied and those of Dr. Oliver and his group may well be dependent upon a difference in altitude. Some of the recent studies going on rather extensively in National Jewish Hospital and at the Medical School in Denver would suggest that there are real differences in the respiratory rate and physiology, even at the Denver altitude of 5000 feet, a fact that we were not sure of before. I thought the comment might be worthwhile that the differences may be partially a matter of altitude and partially a matter of the great differences in the apparatus.

DR. HERBERT C. MILLER, Kansas City, Kans.: I am bothered a little bit by the so-called basal conditions and particularly by the high respiratory rates. It seems to me that with this kind of apparatus there ought to be some effort made to establish the respiratory rate prior to putting the babies into the apparatus, or connecting the baby to the apparatus. I wonder if such estimates were made in these experiments.

DR. MILDRED STAHLMAN, Nashville, Tenn.: I would certainly like to congratulate Dr. Oliver on this very nice piece of work. As far as the respiratory rates are concerned with very small babies, I certainly think that the rates we found were comparable to the type that he got with his apparatus. I think the rates which you obtained with direct measurements of either spirometry or ventilation are somewhat different than those obtained by indirect plethysmography, primarily because I pick up small changes with direct measurements which cannot be recorded with more indirect plethysmography. Dr. Oliver's approach, I think, to this age group is certainly very good. Although I am sure it is not necessary I would like to caution him. He did mention in the abstract the assumption of dead space in the estimation of alveolar ventilation from this type of data. I think it is somewhat hazardous to make the assumption that the dead space to tidal volume ratios will be maintained particularly in children who have respiratory abnormalities, which is the area where this type of measurement could certainly be very valuable.

DR. T. K. OLIVER: Dr. Cook, first, the question of dead space. The measured dead space in the mask was between 9 and 10 ml. However, we used a cushioned mask, and the face of the infant fits into this. It is difficult to tell how much more air is displaced, but the dead space is less than 10 ml. We did use the nomogram for determining body surface area and we are aware of the criticisms, particularly those made by Dr. Karlberg, in this respect. However, in reviewing Dr. DuBois' book on metabolism, published in the 1930's, he discussed this at some length and he pointed out that the nomogram has been applied on a number of infants by several German investigators making measurements on infants. He is quite satisfied that it is as valuable as any other measurement of surface area and just as correct as for adults. I am interested in what Dr. Washburn told us about altitude. Dr. Miller, we do not make a claim for basal conditions. These infants are not in a basal state. They are in a postprandial steady state, and this may account for the fact that the respirations are somewhat more rapid. We, of course, did, as I mentioned in the paper, measure respiratory rate before and after the mask was applied. There was no change. Therefore, I firmly believe that we recorded the true respiratory rate. I would agree with what Dr. Sathlman said about the errors in assuming dead space, and we, for that very reason, did not show data in this regard. We used it only as an interesting tool to play with. We would hope, now that we have collected the air in the bag, that we could begin to make measurements of oxygen consumption and expired CO_2, making correct measurements of dead space. This has been done so ably by both Dr. Stahlman and Dr. Cook. There is one other point regarding the dead space which we tried to indicate in the paper. We were concerned about the risk of a build-up of CO_2 and our tracings, even the prolonged ones of several minutes duration, showed no evidence of an increased rate or increased tidal volume. We believe, therefore, there was no significant rebreathing of CO_2.

21. Study of Juvenile Pernicious Anemia

DORIS A. HOWELL,* Duke
Introduced by LOUIS K. DIAMOND.

An opportunity to study three out of five siblings suffering from classical juvenile pernicious anemia, and to study their parents, grandparents, and more than 50 close relatives, has offered promise of much needed information on this unusual disease of childhood and hope for more definitive evidence as to mode of inheritance.

The three patients, aged 10, 7, and 6 years, of Dutch and Irish ancestry, whose symptomatology dated from the first year of life were subjected to intensive investigation including: Urinary protein electrophoresis and paper chromatography, gastric analysis for free acid and after histamine, vitamin A uptake, radioactive stool fat excretion for neutral fat using triolein, serum iron, formimino glutamic acid excretion, serum B_{12} assay by the

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Euglena and Ochromonas biologic techniques, cobalt[60]-labeled B_{12} uptake measured by blood levels and urinary excretion per the Schilling technique (both alone and with intrinsic factor), gastric cytology by aspiration, and gastric and small bowel biopsies. All three children had been receiving intermittent oral therapy, particularly folic acid, since infancy. When first examined their anemias were minimal but bone marrows were diagnostic. The youngest child showed moderately severe combined system disease of two months duration.

The two normal siblings, parents, and four relatives with symptoms were thoroughly investigated and other available members of the family were tested for presence of anemia and achlorhydria; uropepsinogen and serum B_{12} determinations were performed.

The appearance of this disease in early childhood may represent the homozygous state of a gene for defective intrinsic factor formation or activity. From our own studies on these patients and the reports of other investigators of juvenile pernicious anemia we suspect that the basic defect in this disease is a congenital absence or deficiency of intrinsic factor formation which in turn results in failure to absorb vitamin B_{12}. The combined deficiency of these factors may cause severe alterations in the gastric cells (to be illustrated) which eventually progresses to gastric atrophy and achlorhydria. It is our premise that the achlorhydria demonstrated in adult pernicious anemia patients is a secondary result rather than a primary positive factor in the disease.

Discussion

DR. SANFORD LEIKIN, Washington, D. C.: I would like to ask if B_{12} binding capacities were determined on the blood of these patients or were post-treatment B_{12} levels obtained? A patient has been reported who had no ability to bind B_{12} and who had to subsist on continuous infusions of B_{12} intravenously.

DR. LOUIS K. DIAMOND, Boston: For several years, we have been following three patients with what we thought was fairly classical pernicious anemia of the juvenile type. The oldest patient is now 22 years old. Recently we attempted to reevaluate these three patients through the help of Dr. McGovern of The Massachusetts General Hospital and also through our association with the group at Seattle. The oldest boy is now serving in the Air Force in Germany, and he could not be returned for tests, but the other two patients were retested. To our surprise, they showed some of the strange findings Dr. Howell has reported here this morning. There is a suggestive history of pernicious anemia in the families of both these children. Our first patient is a 12-year-old girl who has a positive family history. She showed that even when given B_{12} with the intrinsic factor she failed to absorb any of it. A 7-year-old boy, the second patient, showed some absorption of cobalt B_{12} when given with intrinsic factor. In addition, his gastric juice, when given to a classical adult pernicious anemia patient in relapse, produced some reticulocytosis. The questions that may be raised are, first, whether this is a variant of pernicious anemia or, second, whether the presence of normal gastric juice and normal small bowel in one patient but almost complete atrophy in the child who was constantly under inadequate treatment means that this might be a progression of intestinal changes leading in later life to the typical pernicious anemia in adults. I should like to ask Dr. Howell, first, if there is any explanation for the finding that the eldest of these three children in her family showed relatively mild change, contrasted to the youngest who had the most severe change. I should also like to ask whether this may not be, instead of a homozygous form of hereditary pernicious anemia, a dominant trait which expresses itself in variable degrees of penetration in the three members of the same family.

DR. JAMES A. WOLFF, New York: I would like to congratulate Dr. Howell for her very interesting study. I think she has answered a number of questions that have bothered many of us and perhaps raised a few more. Some years ago, we reported on two patients with juvenile pernicious anemia. Unfortunately, at that time, we did not have biologic assay of B_{12} activity in the gastric juice, and we have not been able to obtain that since that time. We postulated at that time, because these patients had free hydrochloric acid in their gastric juice, that juvenile pernicious anemia could occur without gastric achylia. One of our patients also had combined system disease, the first time it had been reported at this age. Since then, we have become somewhat critical of our own report and we wonder whether or not with newer biologic methods these patients really would fit the criteria that Dr. Howell has so well shown here. Since then, we have had another patient who has also been studied by gastric biopsy by Dr. Rubin. This patient has complete gastric atrophy as seen in adult pernicious anemia. This patient had a biologic assay and, by Schilling's test with radioactive cobalt, had no intrinsic factor in the gastric juice. Therefore, he seems to fit into the classical picture. Perhaps what Dr. Howell has done is to show, as Dr. Diamond already mentioned, that juvenile pernicious anemia may be a form on the way to true disease and that gastric atrophy as shown in the three patients in this family is secondary to prolonged intrinsic factor deficiency.

DR. ROBERT J. MCKAY JR., Burlington, Vt.: In our patient with combined system disease, whom Dr. Wolff mentioned, the neurologic symptoms were provoked into an acute exacerbation by administration of folic acid. I would like to ask Dr. Howell if this was true of her patient with combined system disease.

DR. DORIS A. HOWELL: We have not assayed B_{12} binding capacity yet. The children have not received any therapy except the 1000μg. flushing dose after each of the cobalt tests. This amount has been enough to take them into hematologic remissions. Dr. Diamond, I am not sure I can answer your questions, but I have a feeling that the difference is so wide among these patients because the eldest child has received a great deal of intermittent intramuscular B_{12}. Unfortunately, the Army took the father, and the family went with him. Every time they moved to another base, the doctors at the new base wanted to restudy all of the children. The younger ones never did receive any intramuscular B_{12} or liver extract, but they did receive massive quantities of oral B_{12} and folic acid. The father finally found an old preparation used on the eldest child and started giving it intramuscularly to the middle child who had the next most severe disease. I am not geneticist enough to be able to discuss the relative degrees of dominant traits with variable penetrance as opposed to the homozygous state, but I suppose, as we advise this family and others, that we should determine more about this. Dr. McKay, I think I answered your question. We have not done any liver testing studies as yet.

22. Adenosine Triphosphatase Activity in the Cell Membranes of Normal and Nephrotic Rat Kidney Tubule Cells

HERMAN W. SPATER * and VINCENT DRAPIEWSKI,*
Albert Einstein College of Medicine
Introduced by HENRY L. BARNETT.

Continuing cytochemical studies of experimental nephrosis have revealed observations which may contribute to our understanding of the pathogenesis of this disease. Recent observations with the electron microscope by Sjostrand, Rodin, Pease, and Ruska have revealed numerous deep infoldings of the plasma membrane into the cytoplasm of the cells in certain segments of the renal tubule of the normal rat. Using the staining method of Wachstein and Meisel, we (Spater, Novikoff and Masek) have found that some of these membranes exhibit high adenosine triphosphatase (ATP-ase) activity in six species studied. Marked enzyme activity, which survives formol-calcium fixation is seen in the ascending loops of Henle of the rat, whereas the membranes of the proximal tubules show ATP-ase activity only in unfixed cryostat sections but not in formol-calcium-fixed frozen sections.

Substitution of glycerophosphate or adenosine-5'-phosphate for ATP in the substrate medium gives no staining of the membranes.

In rats with edema produced by injection of aminonucleoside or antirat kidney antibody the membranes of the proximal tubules acquire high ATP-ase activity which survives formol-calcium fixation. Normal animals injected with DOCA or animals dehydrated by thirsting did not increase proximal tubule-cell membrane ATP-ase activity, whereas injection with pitressin resulted in increased enzyme activity in this region.

The energetics of membrane activity and "active transport" of molecules in the normal renal cells may be related to the juxtaposition of the plasma membranes with high ATP-ase activity and the mitochondria, which synthesize ATP. It is of interest that the enzyme activity of these membranes increases markedly when the metabolism of the renal cell is apparently altered by experimental induction of nephrosis.

Experiments are in progress to investigate whether there is a functional relationship between the increased ATP-ase activity of the membrane and the accumulation of edema fluid.

Discussion

DR. JONATHAN T. LANMAN, New York: I should like to ask Dr. Spater whether the labyrinthine system that he showed so beautifully could be a specialization or adaptation of the smooth reticulum which is seen in all cells under the electron microscope. I should like to ask whether he knows by what means the system can be distended or lapsed.

DR. SAMUEL BESSMAN, Baltimore: These extremely exciting observations by Dr. Spater again bring to light the importance of organization in biochemical reactions. We have a similar membrane question in the diabetes problem in which a question of the effect of hormone on a membrane arises. Here, we have a demonstration, by this kidney preparation, that an organized relation between the generation of energy and transport can exist. The question that I would like to ask is this: insulin is known to have some effect on membrane transport in the kidney, at least on certain substrates. Therefore, I would like to know if Dr. Spater has done anything with insulin in this preparation.

DR. JACK METCOFF, Chicago: Dr. Bonting, working in Dr. Kark's group has been carrying out chemical analyses of isolated cells of the renal tubule taken from patients with a variety of clinical conditions. He suggests that there may be a striking difference between the quantitative

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chemical analysis for a given enzyme and the apparent staining properties which only provide a crude qualitative assay within the tissue. I wonder if Dr. Spater would care to comment on this.

DR. NORMAN KRETCHNER, New York: I would like to ask Dr. Spater two or three questions. I wonder if he would comment on how fixation of the tissue affects his results and whether he thinks that this is actually a different ATP-ase or whether this is some difference in distribution of enzyme. Then, I have another question. I wonder whether Dr. Spater did any quantitative measurements of ATP-ase and if he thought that in the nephrotic syndrome in the rat there was a redistribution of the enzyme within the cellular structure or whether this was, in fact, an increase of enzyme activity within the labyrinths within the membranes. Finally, I would like to ask whether there were any measurements made with and without dinitrophenol and whether any attempt to relate energy systems to the edematous process was done.

DR. KURT LANGE, New York: I would like to ask Dr. Spater if he has any information as to how long the adenosine was present. How long can this type of activity exist? This is a short-lasting process, and the overactivity of the enzyme dies out. The cell has done a good deal of work, and the intracellular damage becomes so severe that all enzymes decline markedly, including adenosine triphosphatase. Finally, the cell damage is so severe that complete collapse of the cell occurs.

DR. HERMAN W. SPATER: These membranes are definitely not the endoplasmic reticulum. In the renal tubule cells, the endoplasmic reticulum has a characteristic appearance similar to other cells with the typical Palade bodies but these do not have it. As to what I think is extending or contracting the labyrinth space, I really do not know. I suspect that water being reabsorbed from the tubule is a good possibility. Again, I hope this does not all turn out to be artifact. In answer to Dr. Bessman's problem, I have not done any work on relations of insulin to any of this material in my preparations. In answer to Dr. Metcoff's comment, I think there may be considerable difference between chemical measurement of metabolites and their histochemical demonstration. It is quite difficult to evaluate both. We attempt to minimize the amount of artifact by using unfixed rapidly frozen tissue in an attempt to localize metabolites qualitatively and quantitatively as they are in life. In some instances, we think we are more accurate than the biochemists; in other instances, not. Dr. Kretchner, as to whether or not this represents a difference in ATP-ase, with one form that survives fixation in the nephrotic animal, and another that does not seem to survive, it is difficult to say. I do not know whether there is a quantitative increase in enzyme which manages to survive the fixing period or whether it is a different ATP-ase. Slater and his co-workers have certainly shown that there are at least four or more ATP-ases. We did not make quantitative measurements of ATP-ase activity. We did use DNP on a few preparations, and it did not seem to accelerate the activity of the enzyme. We used cysteine which did not seem to accelerate the enzyme, and we used p-chloromercuribenzoate which did not seem to inhibit the enzyme. In answer to Dr. Lange, I quite agree that the degree of hyperactivity of the enzyme seems to run parallel to the presence of marked edema and ascites of these animals. When they have had this disease long enough, there are severe degenerative changes with attenuation in the size of the membrane and apparent decreases in ATP-ase activity. However, this seems to be associated also with the loss of the edema and ascites. They do not keep the symptoms permanently.

23. The Distribution of Bromide in Body Tissues

STANLEY HELLERSTEIN*; CAROLYN J. KAISER*; DANIEL DES. DARROW,* and DANIEL C. DARROW, Kansas

Estimation of cell composition directly from tissue analysis or indirectly from dilutional studies is dependent upon measurement of extracellular water. With suitable corrections, chloride space is apparently the best measure of extracellular water. Stable or radioactive bromide is generally used for estimation of chloride space. Because of the slow excretion of bromide, we investigated the use of this anion as a measure of extracellular fluids over prolonged periods.

Normal adults were given potassium bromide by mouth to raise the serum bromide concentration to 2 to 3 mEq. per liter. Urinary losses were measured and serum concentration was followed for periods up to 10 days. The volume of distribution of bromide increased gradually during the first three to four days but thereafter was remarkably constant. After the fourth day, the volume was 14 to 24 ml. per kilogram greater than at 24 hours. In four subjects bromide space remained 197±2.2; 206±1.4; 231±6.6, 232±4.2 ml. per kilogram after the fourth day.

In order to determine the location of the bromide, rats were studied following intraperitoneal injection of this ion. Rats receiving sodium chloride served as controls. Certain tissues, serum, and whole blood were analyzed after 6, 24, 45, and 72 hours. The volume of distribution of bromide was calculated after correcting for red blood cell and

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urinary bromide. The bromide space was 239 ml. per kilogram at 24 hours and 304 ml. per kilogram at 72 hours. Calculating total body chloride from bromide space, these values indicate an increase by a factor of 1.26.

Comparing the ratio of serum chloride to serum bromide with the ratios of these ions in tissues indicates to what extent bromide is concentrated in certain tissues with respect to the concentration in serum. At 24 hours the ratios in serum and tissues are similar. Thereafter, bromide was found in relatively greater concentration in all tissues studied. Calculation of total body chloride from the distribution of bromide in the tissues at 24 and 72 hours indicates an increase by a factor of 1.27.

The data will be discussed with respect to the use of bromide space to measure chloride. In rats, and probably in humans, a 24 hour bromide equilibrium period appears appropriate for estimation of chloride space. After three to four days, bromide space is about 20 ml. per kilogram greater than chloride space and very stable.

Discussion

DR. DONALD B. CHEEK, Dallas, Texas: Over the last five to six years, I have been interested in measuring the bromide space in children. It is important to solve the problem as to what one is measuring with bromide. We did obtain data from rats on total body chloride and found answers which were similar to the total body chloride (exchangeable chloride) obtained from the use of bromide in the same rats. We did not get down to the detailed study of individual tissues that has been presented today. I feel that these data are important because the use of bromide as a measure of chloride is something that is very worth while. I think that bromide as a measure of extracellular space actually offers more than other methods because of simplicity of bromide administration and because our own experiments have defined an amount of intracellular chloride (in the rat). I would like to ask the authors a question. I think, in the second slide you showed a diminution of bromide space at 6 hours and an increase at 24 hours. Am I correct? I want to know how you account for this fall.

DR. GILBERT B. FORBES, Rochester, N. Y.: I would like to ask the author whether he determined the bromide concentration of two other tissues of the body—thyroid and the central nervous system.

DR. STANLEY HELLERSTEIN: Dr. Cheek, it is correct that the 6 hour bromide space in the rats was larger than the volume of dilution at 24 hours. We also found similar alterations in the tissue distribution of bromide at that interval. We have no insight into the physiologic mechanisms involved in these changes. Dr. Forbes, we did not study the bromide content in the thyroid gland or in the central nervous system.

24. A Human Pregnancy Plasma Adrenotropin: Its Identification as Chorionic Gonadotropin and Its Relation to Fetal Adrenal Enlargement

JONATHAN T. LANMAN and JENNY DINERSTEIN,* New York University

Jailer has recently found in the plasma of pregnant women an adrenotropin which is distinct from ACTH in that it does not cause adrenal ascorbic acid depletion. We have wondered if this factor was related to the adrenal enlargement which occurs in the human fetus. The adrenal gland of the human fetus is proportionately 10 to 20 times larger than the adult adrenal. Eighty per cent of the gland is composed of a histologically distinct central reticular zone which involutes postnatally. This zone is the fetal zone; it has recently been shown to be steroidogenic, but the tropic factor responsible for its hypertrophy is unknown. Chorionic gonadotropin (CG) has been suspected; a fetal zone exists only in primates, and primates are the only mammalian order which produces CG.

We have attempted to identify the maternal plasma adrenotropin and have evidence that it is CG. Maternal plasma (27 to 50 I. U. CG per cubic centimeter) given to 40-day-old hypophysectomized male rats (0.3 cc. t. i. d. $\times$ 8 days subcutaneously) partially prevented adrenal involution (adrenal weight: non-pregnancy-plasma-injected controls 14.6$\pm$1.5 mg.; pregnancy plasma 21.0$\pm$2.8 mg.). Chorionic gonadotropin (Ayerst) (15 I. U. per cubic centimeter) did the same (20.6$\pm$1.5 mg.). The minimum effective dose of CG contained 1.0 I. U. per cubic centimeter and of dilutions of maternal plasma contained 1.5 I. U. per cubic centimeter). Both produced adrenal reticular zone hyperplasia and maintained the interstitial cells of the testes in the hyposectomized rats. Neither had ACTH activity when tested by adrenal ascorbic acid depletion, and neither repaired the sudanophobic zone which characteristically appears in the adrenal fascicular zone in the absence of ACTH. The action of CG resembles that of luteinizing hormone (LH). A preparation of sheep pituitary LH (Armour) in dosage equivalent to 8 I. U. CG (rat ovary hyperemia assay) also partially prevented adrenal weight loss (20.8$\pm$2.9 mg.) and produced histological changes in the testes and adrenals identical with those seen with CG and maternal plasma. These data indicate that the maternal plasma adrenotropin is CG.

CG is made in the cytotrophoblast of the placenta; it appeared likely that the CG concentration

* By invitation

in the fetus would be high, thereby explaining fetal adrenal hypertrophy. This assumption proved incorrect. No measurable CG activity (sensitivity limit 2 I. U. per cubic centimeter) was found in early fetal plasma (3rd to 5th month) obtained by ventricular puncture, or cord plasma from normal deliveries or term fetal urine, whereas maternal plasma and sometimes amniotic fluid from the same deliveries had readily measurable amounts. Neither early fetal nor cord plasma prevented adrenal involution in hypophysectomized rats (16.7±2.4 mg. and 15.6±3.2 mg. respectively). Demonstration of an adrenotropic action of CG supports the concept that it is related to human fetal adrenal hypertrophy, but the action is not directly on the gland. An indirect action is being investigated.

Discussion

Dr. Robert Klein, Pittsburgh: I was very pleased with this excellent paper, but very disappointed with the final conclusions. I would beg Dr. Lanman to keep on in this direction because I think that something of the sort must explain the paradox that occurs in the development of adrenal hyperplasia. I think it is clear that the basic difficulty is inability to make hydrocortisone, yet we have pseudohermaphrodites at birth. I think that the lack of F-making ability should in no way stimulate the fetal pituitary to put out ACTH. I think there must be another factor, and I think Dr. Lanman will tell us what it is next year.

Dr. Charles U. Lowe, Buffalo: Dr. Lanman, I wonder if you considered another possible explanation for the phenomenon described. As I followed your first slide, it appeared to me that your rats had a very short period of time following hypophysectomy before you began to administer your chorionic gonadotropin. Sayre, in a series of somewhat elaborate experiments, has demonstrated a competition between ACTH and ADH for receptive sites throughout the body. If I interpret his experiments correctly, one can reach the conclusion that for a period of about two weeks after hypophysectomy that there is still ACTH left in these donor sites throughout the body. What Sayre has demonstrated, I think, is the fact that one must either deplete these sites by the administration of ADH or else wait beyond two weeks. At the end of that time, one has an animal which is not only hypophysectomized, but entirely free of any indigenous ACTH. If any of the material that you injected contained ADH in unusual amounts, it is obvious that it would have had the effect of releasing ACTH which had been in these receptor sites and which is released by the administration of ADH.

Dr. Melvin Grumbach, New York: Would Dr. Lanman consider the possibility that the effect of human chorionic gonadotropin (HCG) on the adrenal gland of his hypophysectomized rats may have been caused by small amounts of ACTH present as a contaminant or through some secondary effect of HCG. C. N. H. Long and his associates at Yale reported several years ago that commercial preparations of human chorionic gonadotropin seemed to contain small amounts of ACTH when assayed by a highly sensitive method. When the gonadotropin was heated at 100 C. for 15 minutes there was complete loss of interstitial cell stimulating activity but the ACTH activity in his preparation was not destroyed. I don't think the failure to demonstrate depletion of adrenal ascorbic acid excludes this possibility since Guillemin has already shown that following administration of small amounts of ACTH to hypophysectomized rats steroidogenesis was caused without a fall in adrenal ascorbic acid.

Dr. Jonathan T. Lanman: In answer to Dr. Lowe's question in regard to the free state in ACTH, we obtained similar results using gonadotropins from three widely different sources—namely, human chorionic gonadotropin, sheep pituitary luteinizing hormone, and equine gonadotropin. It would be remarkable if they were all contaminated to the same degree with some other active principle. Moreover, the presence of the testis is necessary to obtain the observed effect. And lastly, ACTH will not reproduce the histological effects seen in the adrenal. In answer to Dr. Grumbach, Golla and Rice have claimed a similar effect of heated pregnant mare serum on adrenal weight. Their data showed only one insignificant increase with the heated serum. I am not acquainted with Dr. Long's data.

25. Platelet Factor 5: A Newly Identified Function of the Blood Platelets

Irving Schulman; Carl H. Smith; Marion E. Erlandson,* and Jacqueline Wehman,*
Cornell

At present four platelet factors, influencing different phases of the coagulation mechanism, are recognized; platelet factor 1, accelerating conversion of prothrombin to thrombin, platelet factor 2, accelerating conversion of fibrinogen to fibrin, platelet factor 3 participating in the formation of thromboplastin, and platelet factor 4 acting as an antiheparin. Of the known platelet factors, factor 3 is clearly a phospholipid and various cephalins of plant and animal origin have been found capable of substituting for platelets in the formation of thromboplastin.

In testing thromboplastin generation, serum and barium sulfate absorbed plasma supply the non-

* By Invitation

platelet coagulation factors and are commonly diluted in saline, 1:10 to 1:5 respectively, just prior to use. In studies on the use of a soybean cephalin as a platelet substitute it was found necessary to allow the serum to stand diluted for one hour or more in order to achieve the thromboplastic activity obtainable with platelets. The time for maximal activation of the serum varied with different specimens but could be reduced in all by substituting buffer (pH 7.3) for the saline diluent or by freezing and thawing the serum. The increased serum activity on dilution was found to result from reduction in antithromboplastic activity. The different behavior of the same serum with cephalin as compared with platelets suggested a specific function of platelets not possessed by the lipid, namely the ability to neutralize serum antithromboplastin.

To eliminate the possibility that the observed differences resulted from a lesser thromboplastic potency of the lipid, systems were devised in which the initial rate of reaction and yield of thromboplastin was similar using cephalin and platelets. The disappearance of thromboplastin was then followed in each system. A more rapid rate of disappearance of the formed thromboplastin was observed in the platelet-containing system. The fact that the antithromboplastin neutralizing function of platelets was distinct from their thromboplastic potency could be further demonstrated in several ways: (*a*) boiling the platelets destroyed the neutralizing function without altering the thromboplastic potency; (*b*) aqueous extraction of platelets removed the neutralizer leaving full thromboplastic potency in the insoluble fraction.

This newly described function of platelets would appear to have physiologic significance in the hemorrhagic diathesis of thrombopenic and thrombasthenic states. The factor has been designated platelet factor 5.

Discussion

DR. WILLIAM KRIVIT, Minneapolis: This is beautiful work which demonstrates the many functions that platelets have and, therefore, the necessity for using whole platelets when performing thromboplastin generation tests as a screening procedure. Rebuck has recently demonstrated that specific qualitative functions of the platelets are needed for the thromboplastin generation test. I would, therefore, wonder whether it would not be better to use the platelets in the TGT as they come from the body rather than platelet substitutes.

DR. ARTHUR E. MCELFRESH, Philadelphia: I would like to ask Dr. Schulman two questions. One, the order of magnitude of this change appears to be considerable in your graphs. Yet, I could not document this with day and hour. I am sure we have on occasion substituted platelets which have been made freshly that day in our laboratory for stored platelets without finding significant change. Our stored platelets are frozen. I wonder whether you have artificially magnified the magnitude of this change to demonstrate it in this system. I wonder whether you can correlate this with the increasing effect of stored serum as contrasted to fresh serum in the thromboplastin generation. We noticed this particularly working with the serum of newborns.

DR. IRVING SCHULMAN: Dr. Krivit's question, as I understood it, raised the point about why we were interested in platelet substitutes. Did you mean for testing purposes or therapeutic purposes or both? For testing purposes, one of the most difficult problems in studying thromboplastin generation was the ability to get one platelet suspension which was exactly the same as another used previously. One can easily get 100% platelet activity, but attempting to prepare a suspension which has the same potency for experiments over the course of a year is difficult. The search for platelet substitutes is simply for an experimental laboratory diagnostic tool. We felt it was important. Here, we can weigh out a particular amount of platelet activity day by day and know we are dealing with the same type of reagents. I think nobody can deny the need for platelets for therapeutic purposes. The whole question of what makes serum act the way it does is a very tricky thing. You put it in the freezer and take it out a few days later, and you have a different agent altogether. The question has been brought up as to whether new factors appear in the serum or old factors disappear. I personally am being progressively convinced that much of the activity occurring in serum on storage is due to disappearance of inhibitor agents rather than the appearance of new agents or activities or the increase of activity of those already present.

26. Calcification Studies in Clinical Vitamin D Deficiency and in Hypophosphatemic Vitamin D-Refractory Rickets: The Induction of Calcium Deposition in Rachitic Cartilage Without the Administration of Vitamin D

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It is now widely accepted that the clinical response of hypophosphatemic vitamin D-refractory rickets to treatment with massive doses of vitamin D is frequently unsatisfactory. Therefore, during the past four years, we have been examining certain physiological aspects of bone calcification in

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various clinical forms of rickets. In an abstract recorded in the Proceedings of the Society for Pediatric Research, 1956, we reported investigations carried out upon two children with hypophosphatemic vitamin D-refractory rickets who were not receiving vitamin D therapy. The establishment of normal serum concentrations of inorganic phosphorus by continuous intravenous infusion of a suitably buffered isotonic solution of sodium phosphate rapidly resulted in radiographic and biochemical evidence of bone healing. The findings demonstrated that the deposition of mineral in the rachitic cartilage of patients with vitamin D-refractory rickets was not necessarily dependent upon the large amounts of vitamin D ordinarily employed in the treatment of this disease.

Subsequently we have employed similar techniques to study the calcification mechanisms in four untreated infants who were severely rachitic as a result of vitamin D deficiency. The continuous intravenous administration of inorganic phosphate to two patients, and of both phosphate and calcium (alternately) to two, resulted in rapid calcification of the rachitic lesions. New bone mineral was evident radiographically as early as two days after initiating therapy, and in each case the alkaline phosphatase activity decreased gradually during the 6 to 12 days of treatment. Since the deficiency of vitamin D remained uncorrected, the findings suggest that this vitamin is not essential for calcification of rachitic cartilage in the human.

The intravenous infusion of phosphorus is obviously of little practical value in the therapy of vitamin D-refractory rickets. However, we have recently studied the response of a child with long-established untreated hypophosphatemic vitamin D-refractory rickets to a daily oral intake of 1800 mg. phosphorus, given as a supplement to the normal dietary phosphorus intake (1300 mg. per day). The phosphorus supplement was administered as buffered isotonic sodium phosphate in six doses daily; no vitamin D supplement was given. We demonstrated a statistically significant increase in the serum inorganic phosphorus concentration from a mean pretreatment value of 2.4 mg/100 ml. to a mean concentration in excess of 3.7 mg/100 ml. during therapy. The phosphorus balance became positive, and a readily detectable improvement in the rachitic lesions was evident radiographically within one month. Our preliminary findings suggest that oral phosphate supplementation exerts a specific beneficial effect upon the lesions of hypophosphatemic vitamin D-refractory rickets.

Recently, in a patient with hypophosphatemic vitamin D-refractory rickets who was receiving vitamin D at the upper limit of his tolerance, we have observed that supplementation of the normal 1500 mg. dietary phosphorus intake by the administration of 1800 mg. of phosphorus (as inorganic phosphate) resulted in an improvement in the radiographic appearance in the regions of the epiphyseal plates and metaphyses of the long bones. This was accompanied by increases in the retentions of phosphorus and of calcium, and a reduction in the urinary calcium output during the three week period of study. The findings give radiographic and metabolic corroboration to the earlier balance studies reported by Saville et al. (Clin. Sc. 14:489, 1955), and suggest that the administration of supplemental oral phosphate may prove to be a useful adjunct to vitamin D in the treatment of hypophosphatemic vitamin D-refractory rickets.

Discussion

DR. ROBERT KLEIN, Pittsburgh: I would like to ask Dr. Fraser two questions. Does this increase in calcium absorption occur also with citrate administration? I think this demonstration is very important in the case of the phosphate because it presumably occurs through the lowering of calcium and perhaps through other mediums. We have explained our past difficulties with balance studies on this postulate at times, but I did not realize that it had not been demonstrated. The other question I would like to ask is whether this effect occurs in those patients with the unusual forms of rickets which we are all seeing wherein the patient has obvious rickets and grows poorly but where there are none of the serum chemical changes that we ordinarily see. Finally, I think that as far as growth is concerned, it is a matter of when you start treatment. If treatment is started early enough and is successful, these children do grow normally.

DR. ROBERT W. WINTERS, Chapel Hill, N. C.: I would like to congratulate Dr. Fraser on this beautiful and imaginative piece of work. In several of the patients that we have been following, we can fully confirm your conclusions concerning phosphate supplementation orally in the presence of vitamin D, although with somewhat less elegant data. I would like to raise the question about the importance of serum phosphorus. It seems to me that, when these children take the phosphate supplements, the level of phosphorus in serum that one measures is in large part conditioned by the time interval after the preceding dose of phosphate. I think it has been the general experience of many who have treated this disase that hypophosphatemia may persist and that healing occurs in the presence of hypophosphatemia. It seems to me that these points are not at all incompatible with Dr. Fraser's data, and there is theoretical as well as experimental evidence to support this point of view.

DR. MALCOLM A. HOLLIDAY, Pittsburgh: I would like to raise the question as to whether these rather large doses of phosphate could not, in time, lead to kidney injury. Kidney damage has been demonstrated to result from larger doses of phosphate given to rats. If this occurs in experimental animals it would appear necessary to evaluate this factor before these relatively large doses of phosphate are given to humans over a long period of time.

DR. LYTT I. GARDNER, Syracuse, N. Y.: My question concerns only your patient with vitamin D-deficiency rickets. I wonder if you have seen any evidence of tetany in the patient given inorganic phosphate solution. Many years ago Klercker and Odin (Acta Paediat. 5:79, 1925) made extensive studies of the tetanogenic effects of inorganic phosphate solutions on children with vitamin D-deficiency rickets. They found that potassium phosphate solutions caused a more marked increase of muscular irritability than did the sodium salts.

DR. HAROLD E. HARRISON, Baltimore: I am more interested in the theoretical aspect of Dr. Fraser's presentation than the practical treatment, although they are both brilliantly demonstrated in the paper. The question is again raised by Dr. Fraser's paper as to whether the concentration of inorganic phosphate in the surrounding fluid is the sole determining factor in the precipitation of bone mineral in the calcifying zone. There have been fluctuations of ideas back and forth for a period of years. Dr. Fraser is bringing forward again the emphasis on inorganic phosphate. In recent years, we have been working on another aspect and have found, interestingly enough, that we can produce calcification in rachitic cartilage at concentrations of inorganic phosphate so low as to be hardly measurable. Local factors must therefore also be taken into consideration. When cortisone is given to rachitic rats, calcification of the rachitic cartilage can be produced in vivo at concentrations of inorganic phosphate in serum less than 0.5 mg. %. We must assume that there is still the possibility that the local factors may be influenced by vitamin D as well as by the concentration of inorganic phosphate in the surrounding medium. I think so far as the practicality of treatment is concerned, the most important factor is one that has yet to be determined—whether, over the long pull, these patients will do better and grow better than those treated with vitamin D alone. Despite large doses of vitamin D some patients may still grow poorly. This is variable. The question is whether phosphate additions will improve growth rate. In the last two years, we have been using dihydrotachysterol in patients with refractory rickets and the only conclusions we can draw at the moment are that this sterol can be used without toxic manifestations, with good healing of the rickets, and, at the moment, we think perhaps with some acceleration of growth.

DR. SAMUEL KARELITZ, New York: Some time in the past, Dr. E. A. Park and Dr. A. Shohl suggested some studies to me which were essentially concerned with the production of rickets in rats by means of Steenbock's 3143 diet, which contained disproportionately high calcium and low phosphorus, ratio of 4:1. When the rickets was well developed which took several weeks, we added phosphate to the diet to make the Ca:P ratio 1:1. We observed that the stool became white and chalky, that the animals did not eat as well as they had, and that they developed hind limb weakness. Their blood calcium was very low (5+), and the phosphorous rose to about 10. The rats had developed chemical and clinical tetany. The rats were continued on the 1:1 Ca:P diet, and after a while both the blood Calcium and the phosphorous levelled off to normal values. About the same time as the studies were made, an infant with rickets was being treated in the usual manner. Phosphate was added to cod liver oil to try to hasten healing. Within two days the child developed tetany. In both the rats and the child rapid healing of the bones occurred. Long ago Kassowitz had added phosphate to cod liver oil to hasten healing. His patients developed typical phosphorous lines in the bone. I do not think that anything that I have said necessarily applies to the resistant rickets but it does, nevertheless, suggest the possibility that tetany might follow in some instances if children with resistant rickets are given unusual amounts of phosphate.

DR. ALLAN M. BUTLER, Boston: I am very interested in this report, and was hoping that the patients given phosphate without vitamin D did very much better than patients given phosphate with large amounts of vitamin D. Ever since the introduction of the large doses of vitamin D effective in this condition, one has been justifiedly worried about the possible undesirable effect of such vitamin D dosage on renal excretion of phosphate. However, I take it that you have as good results when increasing the phosphate intake with vitamin D as you have without vitamin D. Unfortunately, our experience over the past year in dealing with such phosphate intake has not been as good as yours. We have been able, with increased phosphate intake, to reduce the amount of vitamin D and improve the serum inorganic phosphate levels, but we have not been too pleased with the results as regards maintaining a normal phosphate level and perfect healing. Dr. Klein asked a question about citrates. You may be able to answer it. We have tried citrate in these patients, again getting only equivocal improvement that has not been too impressive. In terms of phosphate producing

tetany, Dr. MacKenzie Walser read a very nice paper at the Young Turks on Monday, demonstrating nicely the ion-pairing effect of phosphate on calcium and hence its tetany-producing effect. However, with low serum inorganic phosphate levels one need not worry about tetany.

DR. BENJAMIN KRAMER, Brooklyn: This is a beautiful piece of work. It again emphasizes the important role which the serum inorganic phosphate level plays in initiating calcification. In ordinary vitamin D-deficiency rickets and in the particular form of refractory rickets which Dr. Fraser and his colleagues are reporting, there is no defect in the calcifying mechanism of the cartilage. I am well aware of the fact that in some cases of rickets healing takes place in spite of the fact that the inorganic serum phosphorus level remains subnormal. This merely emphasizes the complexity of the calcifying mechanism. I think that one cannot speak of cases of refractory rickets as a single entity. There are different degrees of resistance to vitamin D therapy. In at least one such case we administered large amounts of phosphate with huge doses of vitamin D orally with no curative response.

DR. D. FRASER: I am very pleased indeed to have had so much discussion of our paper and to have had remarks from so eminent a group of authorities. I shall attempt to reply to as many as possible of the comments.

First, I should like to emphasize that this work was undertaken as an academic investigation of factors involved in the healing of rachitic bone in humans. Although some of our results suggest that the oral administration of supplemental phosphate may possibly have implications in therapy, we are by no means prepared to recommend this technique as a therapeutic procedure until the data are extended. In this respect, I am very grateful to Dr. Butler for his cautionary remarks. We do not yet know, for example, what may be the long-term effects of the increased urinary phosphate output induced by phosphate supplementation.

In the experiments that we reported, we were primarily attempting to assess the metabolic and osseous effects of correcting the hypophosphatemia. The fascinating experiments reported by Dr. Harrison illustrate very well the importance of other factors in bone healing. Certainly these factors require systematic investigation, especially in some of the less well comprehended types of rickets, such as hypophosphatasia, and the syndromes alluded to by Dr. Kramer and Dr. Klein.

In reply to Dr. Klein's question, we have failed to observe any radiographic or biochemical improvement in two patients with hypophosphatemic vitamin D-refractory rickets who were given oral citrate to the limit of their tolerance for several weeks. We did not carry out metabolic balance studies on these patients. In treating refractory rickets by whatever means, I agree that it is important to initiate therapy at the earliest possible time. However, I do not believe that the therapeutic measures at present available can always be relied upon to result in normal growth, even under optimal circumstances.

27. Studies in the Treatment of Acute Iron Poisoning

THOMAS R. C. SISSON and
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University of Rochester

Studies were made of the clinical, biochemical, and histopathologic effects of ferrous sulphate poisoning in animals, and of the effects of various antidotes. A regimen of treatment for acute iron intoxication is proposed as a result of these investigations.

Preliminary studies demonstrated that oral disodium phosphate duohydrate was effective in reducing the mortality from iron poisoning in rats by precipitating iron in the gut.

Experiments were designed to treat dogs given highly lethal doses of ferrous sulphate. Such dosages resulted in loss of blood volume (plasma), hypotensive shock, and acidosis. Packed red cell volume, serum iron, and serum iron-binding capacity measurements were made before intoxication and during treatment. Twelve dogs maintained on a standard diet were fasted for 24 hours before the intrajejunal administration (under light pentobarbital anesthesia) of 200 or 300 mg. per kilogram of ferrous sulphate in solution.

Three dogs served as untreated controls. Four dogs were treated with intravenous calcium disodium ethylenediamine tetra-acetic acid after a delay of 30 to 60 minutes. This was deliberately executed to imitate the delay expected in the treatment of poisoned children. Two dogs were given $Na_2H_2PO_4$ by gavage, and CaEDTA by venoclysis. One dog was given a transfusion of dog plasma, and two dogs were given intravenous infusions of dextran, in the hope that excess serum iron would be bound to plasma proteins or bound by the protective colloid action of dextran.

There was a rise of packed red cell volume in each dog. Serum iron levels were consistently elevated in all the animals, but were reduced and maintained at lower levels in those treated with CaEDTA alone or with $Na_2H_2PO_4$. Life was prolonged for all but one dog when treatment was given; longer for those who received $Na_2H_2PO_4$ and/or CaEDTA. Plasma and dextrans were considered to be ineffective therapeutic agents. Latent serum iron-binding capacities were rapidly reduced to zero as serum iron levels increased.

* By Invitation

It was apparent that some control and reduction of circulating serum iron was possible by the chelating action of CaEDTA.

Discussion

DR. HUGH E. JOSEPHS, Baltimore: I think it is interesting in these cases that the giving of this much iron apparently by-passes the normal physiological control of iron metabolism. I am not at all sure that there is not some form of mucosa block under normal conditions that controls the iron intake and allows it to enter in physiological proportions. Doses as big as this apparently bypass whatever control there is, not only at the point of entry, but throughout the body. Not only is iron transport completely outside the physiological limits but also apparently the excretion of the iron, at least occasionally, is outside the physiological limits. I have personally seen a case with a very large excretion in urine. Urinary excretion does not ordinarily occur even when large amounts are given intravenously in colloidal solution. In this sort of case, the body has no protection against the iron, so that it is distributed and excreted entirely outside physiological control. I wonder if there has been any study here of urinary excretion or whether there was any injury of the kidneys from possible passage through the urine.

DR. ARTHUR E. MCELFRESH, Philadelphia: I think Dr. Sisson has done a very nice piece of work here. We performed exchange transfusions in a child with an excess of a documented intake of 15 gm. The child did not vomit for two hours after injection. We have had nowhere near the level reported here: the high was 512, I think. However, the thing which discouraged us was the small amount of iron we could get out of this boy by performing an exchange transfusion over a long period of time. We lowered the high serum iron to about 250μg., as I recall, with no evidence that we were drawing iron out of his tissues or doing anything similar. I wonder whether the EDTA dose to this patient would actually mobilize the iron in the tissues or what the effect would be.

DR. RALPH WEDGWOOD, Cleveland: It is a great pleasure to see someone tackle the common problem of poisoning scientifically. I would question the use of lavage that is recommend however. The use of lavage has been recommended generally in the past, but induced vomiting might be a faster and better means of evacuating the stomach, as we have demonstrated in experimental salicylate poisoning. Secondly, in considering the use of antibiotics to prevent irreversible shock, as Fine has done experimentally, one must realize that it is necessary to pretreat with antibiotics. This is an impossible situation in the poisoned child. I would hate to see the "prophylactic" use of antibiotics suggested on such a basis. Indeed, when one considers the bacterial flora of many of our hospitals, one hesitates to suggest the "prophylactic" use of antibiotics in any but a few very specific instances, such as penicillin in rheumatic fever.

DR. THOMAS R. C. SISSON: Dr. Josephs, the problem of mucosal block is one which could probably be argued for some time. I do know that with such excessive dosages as we used, there is apparently a breakdown of any protective element within the mucosa itself, whatever it may be. Certainly, we have felt that with these large doses, there was insufficient necrosis of the mucosal wall to allow passage so quickly of that amount of iron. The effect of the ionic iron itself has something to do with cellular damage, which was not represented by necrosis alone. There may have been a more subtle degree of necrosis. Iron was deposited all over the bodies of the dogs, in every organ, but mostly in the liver, lung and heart. There was none in the brain. In the dog on which we did test for urinary iron, the kidney was not damaged. There was some congestion and some deposition of iron within the kidney cortex. This was not specific in the glomeruli. This seemed to be just an all-over deposition. However, we did not do a muscle biopsy of striated muscle. Presumably, it might have been there as well. Kidney damage was not noticeable in any of the animals, even the one which was killed, although this was Dog No. 13, which apparently would have lived. There, the deposition of iron was without relation to the serum iron levels which, as I say, are probably only indicative levels and not absolutely measurable. Dr. McElfresh, the exchange transfusion, I know, has been tried and in some cases successfully. I am not too surprised that there was a poor removal of iron. It is difficult to measure in the first place, and I think that perhaps the deposition of iron is in a form, presumably ferritin, which is not as easily pulled back from the tissues as some other pigment would be. The effect of calcium EDTA is to form a fairly permanent linkage, once it reaches the tissues. It can, of course, in the presence of another metal, be broken, and substitutions of copper, for instance, for the iron ion can be made. Calcium EDTA, however, is excreted through the kidney, in a combined linkage with iron. We had hoped to be able to measure this in vitro. However, such studies as have been done—the digestion of serum and iron—were most equivocal, and I hesitate to say that we were able to prove that the calcium EDTA link to iron had remained intact in the urine as it appeared to us. Dr. Wedgwood, in the matter of lavage, I think it is well known that most of the children who are being reported in the English literature, have had some degree of

automatic vomiting. By the time the physician sees such a child, he has perhaps emptied the stomach, partially anyway. The purpose of lavage is to assist, if you will, in the vomiting, and perhaps to precipitate what iron may remain in the stomach. When enteric-coated tablets are swallowed, some iron tablets may be undissolved within the stomach. Of course, washing them out or vomiting them spontaneously would be very helpful. However, the purpose of the lavage, primarily, is to leave some of the phosphate within the gut so that it will pass from the stomach into the small bowel and precipitate what iron may be there. Certainly, we can see by jejunal injection that the absorption of iron is just as good lower in the bowel, whereas it was previously thought to take place solely in the gastric and duodenal mucosa. The question of bacterial shock is one that has been identified in animals. I am not sure that it is a factor in children. However, it has been well proven in the dog. The fact that protection has been afforded against the irreversible phase of shock, although not against shock itself, was of interest. I think, in the present very discouraging state of treatment of this condition, that anything which might help to prolong the life, at least for a few hours, will tend to protect against the hazard of shock itself and is of value. The use of an antibiotic is not, I think, to be condemned in such a potentially fatal condition. I hardly feel that we need to be diffident about giving antibiotics in such an emergency as iron poisoning often is.

28. Estriol Excretion in Childhood: A Sensitive Index of Adrenocortical Function

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ALFRED M. BONGIOVANNI,
Pennsylvania

A simplified method for the measurement of urinary estriol is described. It involves enzymatic hydrolysis of estriol glucuronide, extraction, rapid saponification, miniature chromatography, and fluorometry. By this method small (1-2μg.) amounts of estriol are detectable in the urine during the first year of life, and the quantity is found to increase equally in both sexes until adolescence. Children with gonadal dysgenesis excrete normal amounts for age. Normal subjects excrete increased amounts of estriol when stimulated with ACTH. Infants and children with congenital adrenal hyperplasia excrete amounts of the steroid greatly in excess of the normal for adult females, and these levels return to normal when the adrenal cortex is adequately suppressed with cortisone. A child with a virilizing adrenal tumor was found to excrete excess estriol; the urinary level fell upon removal of the tumor. In contrast, no estriol could be detected in the urine of a 13-year-old male with Addison's disease. It is concluded that urinary estriol in children primarily derives from adrenocortical secretion of estrogens. The measurement of this steroid serves as a highly sensitive index of adrenal activity in the preadolescent child.

Discussion

DR. LYTT I. GARDNER, Syracuse, N. Y.: I would like to compliment the authors on their paper. Because of problems of methodology the estrogens have been neglected in the past. Some years ago, Migeon and I studied two children with virilizing adrenal tumors (J. Clin. Endocrinol. 12:1513, 1952). I am delighted to see additional data now coming out. I wanted to ask if Dr. Eberlein had the opportunity to give cortisone to this patient with the very large tumor. Migeon and I measured urinary estrogens, both by bioassay and by fluorimetry. When we gave cortisone there was a really astronomical increase in urinary estrogen as measured by both techniques. We could never explain this and have always been hoping that further studies could be made.

DR. WILLIAM A. REILLY, San Francisco: Again, congratulations to Dr. Eberlein and Dr. Bongiovanni for an interesting development that should prove very practical and helpful to those doing clinical endocrinology, particularly in children. Does Dr. Eberlein note any connection between central nervous system stimulation and estriol production? Occasionally I have noted precocity during poliomyelitis in the brain. This may be ACTH stimulation calling out estriol production in the adrenal cortex. Let me stress, as Dr. Eberlein did, the practicality possible in this test.

DR. ROBERT M. BLIZZARD, Columbus, Ohio: I would like to ask Dr. Eberlein whether he has any preliminary data on whether this product he is measuring is possibly a metabolite of some other steroid or is it actually an estrogen secreted by the adrenal as such?

DR. WALTER R. EBERLEIN: In regard to Dr. Gardner's question, we did not give cortisone to either of these two patients with tumor. Consequently, we did not study the effect of cortisone on estriol excretion. In regard to Dr. Reilly's question as to the effect of central nervous system stimuli on the excretion of estriol, we have not explored this area thoroughly but believe it worthy of study. In these two children with adrenal tumors, emotional stress seemed to cause tremendous amounts of estrogens and 17-ketosteroids to be excreted. The two-year-old child with congenital adrenal hyperplasia was the one who excreted the largest amount of estriol, and she was at the same time the most disturbed child. She was constantly terrified of being in the hospital and being subjected to examination. In regard to Dr. Blizzard's question about whether this is actually estriol, I

think this is certainly an important issue to raise. I should have said in the beginning that this work is really not very original. All we have done is taken the Bauld method for the determination of estriol, what is usually considered specific, and simplified it. I think the criticism might be directed at the Bauld method of estriol instead. The question in any case is not easy to answer because in children the excretion of estriol may be less than 2μg. per 24 hours. To characterize any steroid completely you need at least half a milligram. I suspect, however, that this is estriol we are measuring because the amounts that we find in the urine agree very well with those determined by Drs. Migeon and Gardner, using counter-current distribution, by the elaborate chemical method of Brown.

29. Principles Pertinent to Vaccination of Children Against Influenza

A. V. Hennessy, Michigan

The current experience with Asian influenza has served as a timely reminder that at all times influenza exacts a heavy toll in children. Incidence is highest in children, and fatality in infants is exceeded only by that of persons far advanced in years. Repetitive attacks in childhood may have serious consequences which can shorten the expected life span.

Previous studies have demonstrated that vaccination of children is the only method proven to offer an important degree of protection. The objective of recent investigations has been to achieve by vaccination a broad composite of antibodies reacting with all strains of influenza A and B, like that found in sera of the adults whose resistance is greatest. The same principle of broad antigenic coverage against other respiratory illnesses seems potentially applicable. However, the child has an inexperienced antibody forming mechanism and is concomitantly peculiarly sensitive to the toxic effects of large doses of influenza virus. Children do not readily produce antibody after vaccination unless they have been previously infected with strains of the same antigenic family of viruses, in which case vaccination acts as a "booster" stimulus. This principle, previously established by vaccination experiments with swine and A strains prevalent before the birth of the current crop of children, was reemphasized by the results of a vaccination experiment with Asian strains. A poor antibody response was found after a single dose of standard Asian influenza virus vaccine.

In the past to achieve better antibody levels, and to minimize toxic reactions, reliance was placed on multiple small doses given at short intervals—days or weeks. The new information is that very high antibody levels can be achieved if suitable vaccines are given at long intervals, i. e., months, and that small doses can be utilized for primary or booster inoculations. The experiments to be reported describe the enhanced levels of antibody that result when very small doses of mineral oil adjuvant type vaccines are given first, followed by a standard aqueous vaccine. Maximal antibody levels to strains not previously encountered were attained when two doses of adjuvant type vaccine were given several months apart, each containing a small dose of virus. Double doses of potent aqueous vaccines did not produce comparable antibody levels to the strains that were new in the child's experience. Acceptance of aqueous vaccines has been limited because of the rate of systemic reactions that attends their use. Adjuvant vaccines produce no discernible systemic effects. As a result of these studies the prospect for incorporating in a vaccine small amounts of several antigens which could provide coverage for the major respiratory illnesses of childhood seems bright if suitable materials, and schedules of vaccination are used.

Discussion

Dr. Robert Klein, Pittsburgh: I think this is very interesting and nice work. I have, however, several reservations about it. One, I do not think that our experience with immunization of adults has given us cause to be optimistic about the protection offered. They make antibodies well but still are susceptible to clinical disease. Secondly, I think that the usual adjuvants, and I do not know what one was used here, in mass inoculations give high rates of abscesses and other local and serious reactions. Thirdly, I think that the last slide demonstrates that perhaps a large dose once is as good as smaller doses twice. This may be a practical benefit. I think there is one recent development that may answer some of these problems and certainly has more promise than the present adjuvant materials. Youngner has recently demonstrated that flu virus, among others, can be absorbed on cholesterol and other lipoid substances. This concentrates the antigenic material at the same time that it diminishes the protein concentration of the vaccine. This results in much higher titers in animals without any reaction so far.

Dr. Randolph K. Byers, Boston: We have seen quite a few children this winter with quite severe encephalitis following clinical flu. We have blood samples on a few of them, showing rises in antibody to Asian influenza virus with the encephalitis. Several of these children, I think, are probably permanently damaged, and it would seem to me that this is another factor that is very important in whether or not to try to use these vaccines. We have also been on the lookout for children who might have been damaged from these vaccines as far as the central nervous system is con-

cerned. We have not seen any. They have been widely used around Boston this year. We have also not seen any children with encephalitis who received the vaccine.

DR. ALFRED FLORMAN, Manhasset, N. Y.: This is a stimulating presentation. However, it might be worthwhile to recall that there is no evidence for a viremia in influenza. Consequently, it is not yet clear what role the antibody level plays in the pathogenesis of this disease. Most studies on the effect of vaccination are still mainly concerned with rises in antibody level since such levels can be easily measured. Nevertheless, we cannot avoid the fundamental question—do these vaccines actually protect children? I wonder if Dr. Hennessy would give us some data on the protective effect of influenza vaccine in children who are not in enclosed populations, but in the population at large, for that is the kind of information that the practicing pediatrician needs in order to properly care for his patients.

DR. HORACE L. HODES, New York: The remark I am going to make has nothing to do with the value of vaccination against influenza, Dr. Hennessy, but there is one difference between the statistics which you presented and those reported from New York City. Dr. Greenberg, who is in charge of the communicable disease department in the Department of Health in New York City, a few weeks ago showed figures that brought out the fact that the death rate from influenza among infants was not higher than that in the rest of the childhood period. There was no increase in the death rate among infants, as compared with older children.

DR. LEWIS L. CORIELL, Camden, N. J.: We have also seen encephalitis due to Asian flu. Since last October there have been a number of cases. In talking to others around the country, this is apparently a widespread observation. Some of these cases are quite severe, and there have been deaths. All of our cases were mild. It would be interesting for someone to pull these together and analyze the combined operations. We can speculate about the relationship of Asian flu encephalitis to the encephalitis which occurred at the time of the 1918 epidemic and thereafter for a period of years. We have not seen encephalitis due to the influenza A, B or A′ viruses that have been prevalent for the last 25 years.

DR. REICHELDERFER, Bethesda, Md.: Last April, 1957, we had the opportunity to investigate an outbreak of type A influenza in Arlington in a group of persons who had been vaccinated in 1953 and 1954 with aqueous and adjuvant vaccines of the Cuppett, PR8, and Conley strains. During this epidemic we found that those who had received the vaccines were not protected against the type A Maryland/55 strain of influenza, then epidemic in the county. I would like to know how long these two shots will protect.

DR. A. V. HENNESSY: The adjuvants that we are using are the oil-and-water emulsion and they are the modified or incomplete type of adjuvant. We have given over 80,000 doses of this material to young adults with extremely low rates of reaction. We have given often as many as four doses to a single individual. I know about Dr. Younger's data, and it is very promising. He could show that he also has persistence of antibody for a long period of time. It will be a very useful product. Dr. Byers and others brought up the question of encephalitis. I heard last night that somebody had asked about influenza virus of the brain. That was the first time I know of that it has been, so far, at least, publicly reported this year. It is very difficult, of course, during an epidemic in which 40% to 50% of the population are affected with the disease to separate by antibody those people who come in with encephalitis as a complication of the disease and those who would have had encephalitis anyway. I think the only way you can distinguish is either by direct isolation and studies of individual cases or by showing a marked and compounded rise of encephalitis in general with the same peaking as the influenza curves. Does antibody protect against influenza? I am sure that vaccines protect against influenza if there is antibody present in the secretion and this antibody is probably one of the protective mechanisms. It does appear that they are somewhat relatively correlated to the amount that is in the serum. In regard to Dr. Hodes' problem about the death rates, one of the situations where I think the death rate would be higher is in infancy and later childhood, and, of course, people 60 years old and older. They have actually the highest death rate. However, in Chile, Singapore, and the Philippines, where they had serious outbreaks in comparison with ours, the death rate was very markedly elevated. How long will the protection last? I think that if you have high antibody levels against the family and maintain those as long as that family is prevalent, you will have the protection. We have achieved protection using vaccines which contained 1953 strains or earlier. We used these against the epidemic a year ago, and the protection ratios were the highest we have ever seen.

30. Urinary Excretion and Tissue Storage of Sulfated Mucopolysaccharides in Hurler's Syndrome

MELVIN M. GRUMBACH and KARL MEYER,*
Columbia University

Previous chemical and histochemical studies on the nature of the substance which accumulates in various tissues in Hurler's syndrome suggested the

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presence of acid mucopolysaccharides. Recently two sulfated polysaccharides have been isolated from the urine of afflicted individuals, (Dorfman, A., and Lorincz, A. E.; Meyer, K. et al.) one of which was also found in specimens of liver and spleen (Brown, D. H.; Meyer, K. et al.; Stacey, and Barker).

This report describes (*a*) the renal excretion of mucopolysaccharides in nine such patients from 12 months to 12 years of age, with the characteristic clinical and roentgenographic findings, and (*b*) a mucopolysaccharide isolated from a frozen specimen of liver obtained at autopsy from an additional case. In three instances the pedigree was consistent with the sex-linked recessive form, and in two it was consistent with the autosomal recessive form of the disorder.

From 2-6 days pools of urine, collected from nine patients and examined separately, large amounts of a mixture of sulfated polysaccharides were isolated and two fractions identified by analyses of their chemical and physicochemical characteristics and of their susceptibility or resistance to digestion by specific enzymes. In seven of the nine patients, the major fraction was composed of chondroitin-SO_4B (Ch-SO_4B). A second sulfated polysaccharide was isolated which had the properties of heparitin-SO_4 (Hept-SO_4), and varied in concentration from about 10% to 80% of the predominant fraction. Another patient excreted almost twice as much Hept-SO_4 as Ch-SO_4B. In one patient, only Hept-SO_4 was obtained from the urine. Large quantities of Hept-SO_4 were also isolated from the liver of the latter patient's affected male sibling at autopsy. Although Ch-SO_4B and Hept-SO_4 are normal constituents of connective tissue, neither has been detected in the urine of normal individuals, nor has Hept-SO_4 been isolated from normal liver in significant amounts. There was no obvious difference in the pattern of sulfated polysaccharides excreted by patients with either of the two postulated modes of inheritance. The features of inheritance were consistent with the autosomal recessive form and, in two patients, with the sex-linked form. An abnormal excretion was not found in the parents of two patients who have the autosomal recessive form or in the mother of a male with the sex-linked recessive form. Considerable amounts of sulfated polysaccharides were excreted by the two youngest patients, although in each the skeletal changes were minimal.

In one patient a therapeutic trial with large doses of prednisone did not significantly alter the excretion of polysaccharides.

Because heparin is closely related in structure to Hept-SO_4, tests of blood coagulation, including the thromboplastin generation test were carried out in three patients. No disturbance was detected.

The preliminary data presented in the light of clinical and pathologic studies suggest that Hurler's syndrome is a genetically-determined biochemical disorder of connective tissue which results in overproduction of certain acid mucopolysaccharides and abnormal storage within cells and renal excretion of these polymers. Certain hypotheses concerning the nature of the defect and the possibility that gargoylism may be a heterogeneous biochemical, as well as genetic, syndrome will be discussed.

Discussion

Dr. William A. Reilly, San Francisco: This valuable contribution will be appreciated by many working in this field. I should like to recount very briefly the historical sequences we know of in this disease, the sequence of the knowledge of storage phenomenon. Ten years ago, I think, before this Society, we reported the results of our studies in autopsies on eight cases, with biopsy material on four others, in which we were able to show, by staining methods, that there was a strong suspicion of an excess of mucopolysaccharide stored in nearly all tissues. Our main thesis was that this was a storage disease wherein the material was a complex of protein and glycogen. Now these valuable contributions by Dr. Grumbach and colleagues add to our previous qualitative knowledge more exact qualitative information that this really is a polysaccharide and that it is chondroitin B or heparitin sulfate. This is nearer to the answer, and I think this is well appreciated by many of us interested in the disease.

Dr. Roland B. Scott, Washington, D. C.: I wonder if the speaker would comment upon any observed relationship between the storage products (mucopolysaccharides) and the mental defect which is found in many of these patients.

Dr. Arthur F. Abt, Martinsburg, W. Va.: I would like to ask Dr. Grumbach if he determined the serum mucoproteins and, if so, if he found any deviation in them.

Dr. Andrew E. Lorincz, Chicago: As reported at the recent Proceedings of the Federation of American Societies for Experimental Biology in Philadelphia, we have had the opportunity to study the urine of over 20 persons with this disorder as well as the tissue mucopolysaccharide in two autopsy cases. As has been confirmed by this very excellent report, we also found the two types of patients presented here, that is, (1) those patients that excrete a mixture of chondroitinsulfuric acid B (or β-heparin) and a heparin monosulfuric acid-like mucopolysaccharide, called heparitin sulfuric by these authors, and (2) those patients that excrete the heparin monosulfuric acid-like polysaccharide only. In addition to those two types we have found a third form of Hurler's syndrome

in which the chondroitinsulfuric acid B alone is predominantly excreted in the urine. Now, interestingly enough, contrary to the last statement in the printed abstract of this paper, we find abnormal storage of chondroitinsulfuric acid B (β-heparin) in the liver of this third type of patient. By means of a rather simple turbidimetric technique employing acid albumin, we have devised a screening method for detecting the presence of abnormal polysaccharide in urine that avoids the complicated intricate procedures involved in recovering and identifying these urinary mucopolysaccharides. To date, all of our verified cases of Hurler's syndrome have given a positive test, and as yet, we have had no false positive tests. This screening procedure is not sensitive to the very slight elevations of urinary acid mucopolysaccharides reported by others to occur in lupus erythematosus, rheumatoid arthritis, etc. The amount of acid mucopolysaccharide excreted by persons afflicted with Hurler's syndrome is of a much greater magnitude.

DR. MELVIN M. GRUMBACH: The report of Reilly and Lindsay and their associates in 1949 on the subject of Hurler's syndrome is a classic. It was the first paper to suggest that the storage material was not a lipid but a glycoprotein, and thus it anticipated the results that have been obtained by the use of more refined chemical techniques. Dr. Scott, as the summary table illustrated, mental deficiency is not an invarable finding in this disorder. One of our patients had a normal I. Q. This patient excreted predominantly heparitin sulfate in the urine. Recently, Dr. Meyer received a specimen of brain from a physician in Holland. Preliminary studies have shown that these brains contain a large amount of mucopolysaccharide. Dr. Meyer has identified chondroitin sulfate B and small amounts of what appears to be heparitin sulfate. Dr. Abt, we did not study the serum mucoproteins. The serum cholesterol was not elevated in our patients. In four patients electrophoretic studies of the serum proteins showed a slight to moderate increase in the α- and β-globulin fractions. We have followed the work of Dr. S. Lorincz and Dorfman with great interest. I think their screening procedure should prove of value in early diagnosis.

31. An Unusual Case of Chronic Hypokalemia Associated With Renal Tubular Degeneration

R. J. SLATER; P. AZZOPARDI*; P. E. SLATER,* and A. L. CHUTE, Toronto, Canada

A nine-year-old boy has been studied because of polydipsia, polyuria, a craving for salty foods since early childhood and generalised weakness accompanying any moderately severe intercurrent infection. Physical examination was negative, the blood pressure was 90/50. There was no history of preceding renal disease nor of familial periodic paralysis. A sister, believed to have similar complaints, died at three years of age.

Laboratory investigations revealed a consistently alkaline blood (pH 7.5-7.6); usually low serum sodium levels (127-140 mEq. per liter); consistently low serum potassium levels (2.5-3.0 mEq. per liter); usually low serum chloride levels (75-90 mEq. per liter), and slightly elevated bicarbonate levels (28-31 mEq. per liter).

The urine was consistently alkaline but otherwise normal. The specific gravity ranged from 1.010 to 1.015. After two units of vasopressin (Pitressin) given subcutaneously, the specific gravity increased only to 1.018, and no decrease in urine excretion occurred. However, after a concentration test the specific gravity increased to 1.022. Urea and creatinine clearances and the intravenous pyelogram were normal.

Urinary aldosterone excretions on two occasions were 14 and 16μg. per day. The excretion of 17-ketosteroids and 11-oxysteroids was normal.

The sodium and potassium balances were studied during (*a*) a control period of 10 days (intake: Na—97 mEq. per day; K—77 mEq. per day), (*b*) a period of 10 days of moderate potassium supplement (K—123 mEq. per day) and (*c*) a period of eight days of high potassium supplement (K—233 mEq. per day). During these three periods the potassium balance remained negative, and during the first two periods the sodium balance was positive. The chloride balance was positive throughout. The urine remained alkaline throughout, although the serum pH became normal during the period of high potassium supplement. Although there was considerable fluctuation in serum electrolyte concentrations, the serum sodium and potassium levels remained decreased. During the potassium loading in Period 3 the C_4C_{17}% rose to 130.

In order to rule out the possibility of an unusual type of primary aldosteronism, a laparotomy was performed; the adrenal glands appeared healthy. To rule out hyperfunction, the left gland was removed for bioassay; it weighed 3.7 gm. and revealed some fatty infiltration of the cortex on microscopic examination. Biochemical analysis of a section of muscle removed at operation revealed a decrease in intracellular potassium of 20 mEq. per kg. and an increase in intracellular sodium of 20 mEq. per kg. of tissue. In the presence of a normal total body water and a normal extracellular chloride space, the exchangeable sodium was estimated to be 130% of normal.

A renal biopsy specimen revealed areas of marked degeneration of the tubules. These changes were characterized by loss of epithelial cell architecture as well as by dilatation of the lumina by

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eosinophilic, homogeneous material. A few glomeruli appeared hypercellular but were otherwise normal. There were no signs of inflammation.

Consideration of this unusual combination of features raises the possibility that the potassium losing state in this child might be caused by a renal tubular defect rather than occurring secondarily to hyperaldosteronism.

Discussion

DR. WILLIAM B. WEIL JR., Cleveland: It is interesting to notice that this child had a persistently alkaline urine and remained persistently alkalotic as far as the serum was concerned. If this is occurring on the usual diet which normally results in an acid urine, one would have to postulate that there was an unusual cation in the urine or that an unusual absence of bicarbonate would be required in order to keep the child's acid-base balance stable. I wonder whether any studies of the urine, as far as electrolyte composition is concerned, were made in an attempt to answer this problem.

DR. T. H. SHEPARD, Seattle: In reference to the patient that Dr. Holman was discussing, we found in studying the boy that his blood carbonic anhydrase was between 1/2 and 2/3 of that of 25 normal patients. I have always been anxious to analyze this boy's kidney for carbonic anhydrase, but it was never possible. I think it would be interesting to measure the patient's blood carbonic anhydrase.

DR. JACK METCOFF, Chicago: There is a remarkable similarity between the manifestations of this patient whom Dr. Slater has described and the classical description of the Lignac-Fanconi-Debre syndrome, or crystine storage diseases. Several patients with cystine storage diseases have been studied in considerable detail and have been found to have almost all of the features Dr. Slater indicated and to be renal potassium losers with associated depletion of muscle potassium as well (Metcoff, J., J. Clin. Nutrition 4:543, 1956). Ordinarily, cystine crystals may be found in the tissues and are pathognomic of this genetic molecular disease. Crystals may be demonstrated in the conjunctiva, they might possibly be seen on slit-lamp examination, and they may be found in the bone marrow, and possibly in renal biopsy specimens. However, it is essential that the tissue not be fixed in formalin since cystine is soluble in formalin and crystals would not be found under such conditions. I wonder whether search was made for these crystals.

DR. ROBERT W. WINTERS, Chapel Hill, N. C.: I would like to ask if you have any measurements of the urine pCO_2 on this patient.

DR. SAMUEL J. FOMON, Iowa City: We have not observed rapid rates of excretion of ammonia in association with alkaline pH of the urine. I wonder if you would tell us what the rate of excretion of ammonium actually was.

DR. EDNA SOBEL, New York: I am very baffled by the high aldosterone output, as I am sure Dr. Slater is. One would expect persistent hypokalemia to reduce adrenal function in this respect rather than to stimulate it. The sodium concentration of the extra cellular fluid seemed to be less reduced than that of potassium. Again, it is very difficult to account for what might be secondary hyperaldosteronism. Dr. Clark West and I saw two children in Cincinnati, siblings, who had a very similar situation. Normal concentration of potassium in the plasma never was achieved, despite administration of large amounts of potassium chloride. The possibility of increased aldosterone output can be judged, since frozen urine from one of the patients is still available. Their kidneys, which were unfortunately studied at autopsy, showed a similar lesion. The adrenals seemed normal. I wanted to comment on this because it seemed that the children may have died because of an inability to conserve water. They became dehydrated very rapidly during febrile illnesses, and they died unexpectedly.

DR. WALLACE W. MCCRORY, Philadelphia: We have observed a child aged 12 years and a sibling aged 8 years with what is probably the same syndrome. I think there is suspicion in the minds of many people with patients like this about the primacy of the events—whether renal disease came before the alteration in the potassium metabolism. I believe Dr. Slater is describing a patient who may well present a primary abnormality in renal potassium metabolism. Of the patients that we have studied, the 12-year-old boy is capable of responding perfectly normally with urinary acidification to ammonium chloride loads. He had normal inulin and PAH clearances, normal TM PAH, and concentrated urinary solutes to over 1100 mOSM per liter. He has no evidence whatsoever of renal disease. We took note of the report in *Science* (September 1957) in which Liddle and Kagawa called attention to a compound (Spiralactone SC-8109) that may act as an aldosterone antagonist. We obtained some of this through the courtesy of Dr. Bongiovanni. We administered 900 mg. of this compound over a 24-hour period to the patient. There was a decrease in urinary excretion of potassium. I would like to ask whether Dr. Slater had any opportunity to make any such observations on this patient, and also what evidence does he have of the ability of his patient to put out an acid urine when put under stress. I have been led to understand that this compound (Spiralactone) when given to patients with hypokalemia and alkalosis can correct these disturbances.

DR. ALFRED M. BONGIOVANNI, Philadelphia: Was the patient normally developed for his age?

DR. WILLIAM A. BRODSKY, Louisville: Have you speculated on the significance of increased ammonium output in the presence of an alkaline urine? To my knowledge, this has only been seen before in alligators.

DR. R. J. SLATER: In response to Dr. Foman, may I say that Dr. Medovy and I were afraid to attempt to treat the boy by a low sodium intake in view of the chronic hyponatremia. At the time we were unaware that other children with a similar condition were under observation elsewhere. In view of the comments about the success of low sodium intake, I think this is certainly our next step. Dr. Weil, I cannot answer all your questions about the urine electrolytes since they have not as yet been completely calculated. The ammonia excretion varied between 20 and 30 mEq. per liter of urine and the bicarbonate concentration varied from 4 to 20 mEq. per liter. I have not yet calculated the pCO_2 values. Dr. Metcoff, the Fanconi syndrome was considered as a possibility. Dr. Donald Fraser and I could not find any crystals in the cornea of the eye, although slit-lamp examination was not made. I mentioned previously that no evidence of abnormal aminoaciduria was found. Dr. Bongiovanni, the boy is normal in size and weight for his age. I do not know how to answer Dr. Brodsky's questions since we have not carried out any studies on alligators. We considered the increased ammonia excretion to be a reflection of the hypokalemic alkalosis caused by the aldosteronism since this has been the usual finding in this type of patient. Possibly our interpretation is not correct. We intend to complete the studies of the sulphates, phosphates, etc. in the urine, and we may understand better the mechanism of ammonia excretion when this work is complete. Dr. Sobel has asked why the aldosterone excretion is elevated. These studies were carried out on three occasions by Dr. Jacques Genest and on two occasions by Dr. Alan Gornall using different techniques, and in every instance they were elevated above normal. I do not understand the mechanism of this alteration, but it did not appear primary. In answer to Dr. McCrory, we did not attempt to assay the ability of the kidney to acidify the urine by means of ammonium chloride. During the period of high potassium chloride intake, the pH of the blood did decrease to the normal range. However there was no concomitant change in the urine, the pH of which remained alkaline. There was one comment that I wanted to make and it is that I feel in agreement with President's remarks of yesterday that this type of case epitomizes the 'inadequate information syndrome.'

32. A Simplified Test for Histoplasmosis Utilizing Precipitin-in-Gel Reactions

DOUGLAS C. HEINER,* Arkansas
Introduced by THEODORE C. PANOS.

The diagnosis of histoplasmosis may be established with certainty only by demonstrating tuberculate chlamydospores of Histoplasma capsulatum isolated from patients. A presumptive diagnosis is possible by demonstrating intracellular yeast forms on smear or biopsy specimen of infected organs or sputum. Unfortunately it is difficult to make a diagnosis by these methods, except in chronic pulmonary or disseminated histoplasmosis. Therefore, one frequently must rely on immunologic tests. The skin test is a useful indicator of current infection only when conversion from a negative to a positive reaction is demonstrated. Complement-fixation reactions have the disadvantages of cross reactions, a need for shipment of specimens, and the impossibility of performing the test when serum is anticomplementary. Conventional precipitin reactions and colloidal agglutination tests have had clinical trial but have equally important disadvantages. A simple reliable test apparently devoid of these disadvantages has been developed during the past 15 months using the principles of precipitation in gel described in 1905 by Bechhold and improved during the last decade by Ouchterlony, Oudin, and others. The groups studied are summarized with results in Tables 1 and 2.

At least six distinct precipitin bands representing six different antigens and corresponding antibodies have been identified on testing various sera with crude culture filtrate prepared from H. capsulatum. No serum has been responsible for more than four bands. All sera from patients with proved histoplasmosis, however, produced at least two bands. The *m* band, produced by nearly all sera which form any precipitate, is designated *m* because it may readily be caused by histoplasmin

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TABLE 1.—*Precipitin Reactions in Agar Using Crude Histoplasmin*

Diagnosis	Patients No.	Cases with Precipitin Reactions No.	No. with Specific Bands M	H	X	Y	C	N
Histo. proved by culture	7	7	7	7	2	2	1	0
Histo. suspected	257	65	65	20	0	0	0	1
Hosp. survey (suspects excl.)	1728	31	20	8	0	0	1*	2†
Normal controls	34	0	0	0	0	0	0	0

* This serum also contained M and H antibodies.
† Out-of-state patients, not in survey.

TABLE 2.—*Precipitin-in-Agar Reactions Using Crude Histoplasmin, Blastomycin, and Coccidioidin Antigens*

Diagnosis	Patients No.	No. with Specific Bands						
		H. Capsulatum					B. Dermatitidis	C. Immitis
		M	H	X	Y	C		
Histo. proved by culture	7	7	7	2	2	1	1*§	1*
Blasto. proved by culture	13	2	0	0	0	0	5	1
Cocc. Seropos.‡	7	0	0	0	0	0	0	7
Normals before skin tests	22	0	0	0	0	0	0	0
After 1-3 positive Histo. skin tests	7	6	0	0	0	0	0	0
After 3-6 negative Histo. skin tests	15	0	0	0	0	0	0	0

‡ Sera from Dr. C. E. Smith, School of Public Health, University of California.

*§=identical antigen found in H. capsulatum, B. dermatitidis, and C. immitis.

(*mycelial antigen*) skin tests in normal sensitized adults. The *h* band is named because it appears to be specific for *histoplasmosis*. It is not influenced by skin tests. The *x* and *y* bands were observed only twice, and their significance is not established. The *c* band represents a *cross reaction* seen in certain patients with histoplasmosis when their sera are tested with histoplasmin, blastomycin, or coccidiodin antigens. It has been possible to demonstrate that the *c* antigen is a product of Blastomyces dermatitidis and Coccidioides immitis as well as H. capsulatum. The *n* band was named because of its *nonspecific* nature. It is not produced by sera from cases with proved histoplasmosis. Anti-*n* activity was found in only three out of more than 2100 sera. All three of these patients had pica pulmonary disease and marked eosinophilia. Sera of other patients with the visceral larva migrans syndrome failed to produce this band.

Sixteen sera producing both *m* and *h* bands in agar were tested by complement-fixation titers and three failed to have a titer of 1:8 or greater. One of these sera was anticomplementary, the other two had titers of 0. Two, however, had culturally proved active histoplasmosis and the other had yeast forms seen on bone marrow smear, demonstrating the possibility of false negatives in complement-fixation tests. False negative precipitin reactions in agar have not been encountered insofar as none of 24 patients with a positive complement-fixation test, or of seven with proved histoplasmosis, has failed to shown an *h* or *m* band or both. False positive reactions by complement-fixation tests have been seen following histoplasmin skin testing of normal sensitized medical students and house officers. In contrast false positive precipitin reactions in agar have not occurred, in that all such normals have shown only an *m* band, clearly separating them from the proved cases. Fractionation of serum by means of continuous-flow electrophoresis has shown *h* and *m* bands to be produced only by γ-globulin fractions, indicating the likelihood of true antigen-antibody reactions.

The absence of a precipitin reaction in agar provides strong evidence against the diagnosis of active histoplasmosis. A presumptive diagnosis of histoplasmosis is made on the basis of either an *h* or an *m* band in the absence of recent histoplasmin skin tests, or on the identification of both *m* and *h* bands regardless of skin tests.

The simplicity of performance, reliability, and sensitivity of this test make it a valuable tool in the study of histoplasmosis. It is adaptable to routine use in any laboratory; a result may be obtained within 24 to 48 hours.

Discussion

DR. AMOS CHRISTIE, Nashville, Tenn.: Dr. Heiner's paper is of the greatest significance to all of us who find ourselves working with histoplasmosis in the endemic area. I suspect from my correspondence and telephone consultations that it is important to many of the rest of you who have unexplained pulmonary infiltration and hepatosplenomegaly. Dr. Heiner has highlighted the diagnostic dilemma which we have. The skin test, of course, has limitations, which he pointed out, because even in the disseminated disease, only about 50% of the cases will have positive skin test. It has the same significance as the tuberculin test in the very young or those with overwhelming infection, but the cultural methods also have limitations. It is very difficult to grow this organism. It was not until we learned to enrich the media that we began to accomplish this with any ease. We thought we were really inhibiting pyogenic organisms, by adding antibiotics such as streptomycin but we were really enriching the media. We learned that the antibiotics are necessary for the growth requirements of the organism. The therapeutic implications, I think, are obvious also. It leaves you with an unexplained disease and without a sharp diagnostic method. That is why a number of workers, including those in our own laboratory, have been flirting with different serological techniques for a number of years. The fact that we have not arrived at the ultimate answer in our laboratory has not disturbed me because I am reminded by Charlie Smith that he did 23,000 complement-fixation tests for coccidioides before he published his first paper about the technique. In our laboratory, we have been using the complement-fixation test and experimenting with other tech-

niques, but not this one which Dr. Heiner presented so very well. We have been using hemagglutination and senstized red-cell techniques. At the present time, we are satisfied with our complement-fixation test. We are using two antigens. We use both the yeast-cell and mycelial antigen. In the last 237 cases we tested, the results were as follows: 31 were positive to the yeast cell antigen; 71 were positive to the mycelial antigen, and 16 were positive to both. It has a very high correlation with the clinical disease and we can repeat it. Concerning the anticomplimentary reactions, Dr. Heiner has avoided this by his technique. If you use dry syringes and take fasting blood, you cut that down to a minimum with our antigen. We can quantitate our complement-fixation, and we can study a progression or regression of disease by this means. It is an acute phase reaction, and I think that besides the diagnostic significance which it has, it may be, in the final analysis, of greater prognostic significance as it fades out and the disease becomes inactive. In this way, it would be helpful to the clinician. Dr. Heiner's method seems to have fulfilled the criteria of any serological technique. It is sensitive and specific. He assures me it is adaptable and that we can do it in our laboratory. He has certainly made a contribution to the study of this disease.

Dr. Alfred Florman, Manhasset, N. Y.: I would like to bring attention to another simple serological test for histoplasmosis. That is the use of so-called latex particles in place of collodion particles in the collodion agglutination test. This is an excellent test but unfortunately it is not easy always to make collodion particles of the right size and to preserve them. On the other hand, latex particles, which are really polystyrenes, are a by-product of the latex industry, and so are now easily available. They have been used, as you know, for some years in a test for rheumatoid arthritis and we have used them recently in studying bacterial antigen-antibody systems. Within the last couple of weeks, a report has appeared from Saslaw's and Carlisle's laboratory at Ohio State in which they have been able to show positive tests with latex particles sensitized with histoplasmin and human sera. I wonder if you have had an opportunity to examine your sera using this technique?

Dr. Arden C. Miller, Kansas City, Kan.: Dr. Heiner, can you tell us anything about the presence of the F-band in the seven patients who had proven histoplasmosis? How early did this band occur, and how long did it persist?

Dr. Douglas C. Heiner: Dr. Christie, these techniques have proved useful in a number of circumstances. Of the patients on whom we did precipitin tests in agar and got a positive reaction, four had negative skin tests. Two were on steroid therapy at the time skin tests were performed. One of these previously had been shown to have a positive histoplasmin skin test which became negative on steroids. The other one had multiple calcifications in the lungs. A third had disseminated histoplasmosis which was proved on culture and smear of bone marrow. The fourth had early histoplasmosis clinically. In fact, a week later he developed a positive skin test and also had *m* and *h* bands on precipitin testing. As far as semiquantitative comparison of the precipitin and complement-fixation tests is concerned, it is perfectly possible. We can take sera from these patients and make serial dilutions in order to determine precipitin titers. In the patients who have positive precipitin tests and also positive complement-fixation reactions, the titers are usually quite similar. For example, if we make serial dilutions of a serum which has a complement-fixation titer of 1:128 we may get a precipitin titer of 1:128, 1:64, or 1:256. Dr. Florman asked about latex particles. We have not used these at all. I think the disadvantage, at least theoretically, of using latex particles in something as complicated as histoplasmosis is illustrated by the different precipitin bands we have demonstrated. There appear to be at least six different antigens in the histoplasmin preparation that we have made, and any of these may enter into antigen-antibody reactions. Any antigen, I would assume, could adhere to latex particles and could therefore result in a positive test. We feel that we would not have a more specific test with latex particles than we would with complement-fixation tests, although it is true that latex particles might make a more sensitive test. We were looking for increased specificity rather than increased sensitivity. I was asked about the persistence of *h* antibodies in the proved cases. Most of our proved cases, as I said, had chronic pulmonary histoplasmosis and they were impossible to observe in convalescence. Therefore, we have not seen the *h* antibody disappear from sera of these patients. We have followed a small number of patients whom we think clinically had acute histoplasmosis. In some of these the *h* antibody disappears within one or two months after clinical evidence of disease activity is gone. We feel that this follows closely what may be observed with complement-fixation tests in some patients with histoplasmosis following infection.

33. The Response of the Newborn to Major Surgery: Urinary Electrolyte, Nitrogen, and Water Losses

Eleanor Colle MacLean * and
Elsa Proehl Paulsen,*
Minnesota

Introduced by John A. Anderson.

Observations were made on fifteen newborn infants who underwent corrective surgery in the

* By Invitation

first four days of life for tracheoesophageal fistulas, for obstructive anomalies of the gastrointestinal tract, or for meningoceles. Measurements were made of the intake and urinary and gastrointestinal losses of sodium, potassium, chloride, nitrogen, and water.

Sodium ion was administered at maintenance levels of 1 to 2 mEq/kg/24 hr. In addition, variable amounts were added equivalent to the losses in gastrointestinal drainage. In the majority of infants positive balances of sodium and chloride were observed, though in several, negative balances of varying intensity were seen the first 24 hours postoperatively. The absolute amounts of sodium and chloride excreted were slightly greater than in a group of control infants, and consequently the balances, though positive, were small. One in whom sodium and chloride losses were massive throughout the three day study period was subsequently found at autopsy to have changes in the pancreas with cystic fibrosis. Potassium was administered at levels of 1 to 3 mEq/kg/24 hr. In ten infants it was possible to maintain small positive balances at this level. The negative balances in four were small, and they were encountered with one exception when the intake was 1 mEq. or less. The exception was an infant in a state of severe hyponatremia, hypochloremia, and metabolic alkalosis. Urinary K losses were very large, and 4 to 8 mEq/kg/24 hr. were required to maintain a small positive balance. The abnormal serum electrolyte picture persisted for 24 days, until it was possible to introduce oral feedings.

Fluids were given in amounts of 1000-2000 ml/square meter/24 hr. plus a volume for volume replacement of gastrointestinal drainage. Urine volumes were large and ranged from 500 to 1600 ml/square meter/24 hrs. A limited ability to increase osmotic concentrations postoperatively was seen, and this was at times accompanied by slight but definite rises in serum concentrations of sodium and chloride. The large urine volumes may, therefore, reflect in part a diminished capacity to conserve water. Two infants who had small urine volumes (50 to 200 ml/square meter/24 hr.) also had very small urinary excretions of sodium, potassium, and chloride. As a consequence, the serum concentrations of sodium and chloride rose. A third infant was inadvertently given isotonic sodium chloride the first postoperative day and ⅔ isotonic sodium chloride on the second. Urinary excretions of electrolytes, nitrogen, and water were exceedingly small, and serum concentration of Na and Cl became markedly elevated. Maximal urine osmolarity was not achieved until two days after the load was given.

Nitrogen losses postoperatively were greater than those in a control group of normal infants fed glucose and water orally.

Under the conditions of this study the majority of newborns undergoing major surgery in the first four days of life had a postoperative response significantly different from the one generally described in adults. Though potassium and nitrogen losses in the infant and adult were of the same relative magnitude, the infants showed no evidence of postoperative water or sodium retention or hyponatremia.

In fact, the infants had somewhat greater than normal urinary excretion of electrolytes, moderately large urine volumes with low osmotic activity, and normal or slightly elevated serum sodium and chloride concentrations.

Discussion

Dr. Charles L. Fox Jr., New York Medical College: This paper is extremely interesting. I would like to add that I do not think it is entirely fair to say that their results with the infant group are different than what others find with the adult group in terms of the retention of sodium. These investigators have wisely selected a solution in which the total cation concentration is at least twice the chloride concentration, if I understand it correctly. With such a solution, similar results are obtained in adults. All studies with this type of balanced polyionic solution should be sharply distinguished from experiments in which 0.9% saline is utilized, or a solution in which the Na plus K equal the chloride concentration. There is another point I would like to ask about. Was any study made using a solution somewhat less dilute than this, in view of the large urine (water) excretions which were observed?

Dr. Charles U. Lowe, Buffalo: I share with Dr. Fox a reservation concerning the observations in adults. I think the material presented on adults who have undergone surgery was from studies where the solutions were between three and five times as concentrated as those used in this study. I think the lesson for pediatrics concerns not a relationship to adult work, but the prohibition which is still so commonly presented concerning the administration of parental fluids to infants undergoing surgery. I know that Dr. Paulsen was in Minnesota when she did these studies. Even at the time I was there, it was the accepted sense of the surgeons that newborns undergoing surgery should have no fluids for as long as three or four days. I think that these studies successfully refute this point.

Dr. Robert Klein, Pittsburgh: Dr. Paulsen was kind enough to send us urine that she could spare from some of these babies, and we tried to see if we could find salt-losing hormone there. I must admit that we did not get very good results, but she tells me that the one time we said we thought

there was a large concentration of this material in the urine, the baby was the one with cystic fibrosis who had a massive diuresis of sodium. I cannot entirely answer her question about cystic fibrosis but we have tried to see if we could find this material in patients with this disease because of their well-known difficulties. We have not been successful, although we have now been stimulated to go back and try ACTH. Patients with pancreatic fibrosis, put on a restricted sodium intake, restrict sodium output in the urine in a normal fashion.

DR. PHILIP L. CALCAGNO, Buffalo: I am very delighted to hear this presentation today because several years ago we studied the renal diluting and concentrating mechanism in children requiring surgical intervention. We did not study infants in the newborn period. However, the findings that were presented today are very comparable to the findings of Drs. Rubin, Mukherji and myself in regard to the renal water excretion and renal concentrating capacity following surgery. No defect was found with either mechanism following surgery. Secondly, we also carried these studies through to the excretion of a hypertonic sodium chloride solution, postsurgically. Here again, in general, the hypertonic sodium chloride solution was excreted as well in the postsurgical period as in the presurgical period. Three of the group studied were not able to excret sodium in the same manner, but gastrointestinal losses accounted for the differences.

DR. SOLOMON A. KAPLAN, Brooklyn: Some time ago, Dr. Rose Ames showed that the newborn is rather subnormal in its ability to respond to the challenge of a water load. She showed that the urinary output of nonoperated children, boys and girls, in the first few days of life was very much subnormal. I was very taken back to see in one case, an output of 1600 ml. per square meter. Our residents are cautioned to reduce fluids postoperatively in these children because of Dr. Ames' work. I wonder if Dr. MacLean would tell us exactly how often she came up with this large figure of 1600 ml. per square meter per 24 hr.

DR. NATHAN B. TALBOT, Boston: I wonder if she would also tell us whether these babies, in contrast to the adults studied, received substances which may have at times during the immediate postoperative period a considerable influence on water metabolism.

DR. LYTT I. GARDNER, Syracuse, N. Y.: I share Dr. Lowe's warm admiration for this study. I would like to know if either Dr. MacLean or Dr. Paulsen made any measurements of urinary corticosteroids, particularly in children postsurgically.

DR. WALLACE W. MCCRORY, Philadelphia: I think it is very refreshing to see what pediatricians consider reasonable amounts of electrolytes and fluid given to a group of newborn infants postsurgically without any evidence of resultant deterioration of the patients. There is a monograph that I am sure many people here have seen which tends to overstress the fact that, on considerably lower fluid intakes and without potassium supplements, patients can also survive surgery. The work of these authors calls attention to the difference between retention and abnormal retention. Dr. Fleischer, working in our laboratory, has been studying the postsurgical response of young infants to water loads. He has found that some of these infants respond perfectly normally within the first six hours of the postoperative period. He also found that some of them do show an antidiuresis postoperatively that cannot be attributed to dehydration or depression in glomerular filtration rate. Sodium and water retention have been somewhat overstressed. While the patients may show retention in the first 24 hours, the authors have demonstrated the importance of not confusing this with the behavior and requirements of the "recovered patient," i. e., from the second day on postsurgically. One observation I find of particular interest is the patient who, in spite of daily potassium supplements in the range of 4 mEq. per kilogram orally, continued to demonstrate potassium depletion and did not retain the potassium. I wonder if the author could comment on the possible significance of this.

DR. PAULSEN: In this study the amounts of electrolytes and water were kept to what was felt to be physiologic, and no attempt was made to test the upper limits of tolerance. The work had its genesis in our concern over the use of small volumes of 5% dextrose in infants in the postoperative period in the same manner as surgeons use it in adults. They do this in an effort to avoid the effects of salt and water retention they have observed in adults postoperatively. It is certainly possible that larger amounts of electrolytes would have revealed different excretion patterns in these infants. The water retention noted in adults, however, has been shown by LeQuesne in England to be independent of the electrolyte content of the infusion since it occurs with the administration of 5% dextrose as well. From Ames' study one can infer that infants handle a water load poorly. However, she gave infants a water load of ⅛ to ¼ of their daily fluid requirements over a 10 minute period by gavage. The circumstances of our study are considerably different than hers, because the infants in our study received a constant infusion over a 24 hour period. Dr. Barnett has also shown that an infant receiving an excess of water in the latter fashion is able to dilute his urine normally and to the same degree as the adult. Dr. Talbot, the infants did not receive any preoperative

medication such as morphine. They received cyclopropane or pentothal and nitrous oxide. It is possible, on the basis of Dr. Jack Crawford's recent findings, that the administration of pentothal prevented a drop in glomerular filtration rate and therefore a diminution in urine volume.

Dr. Gardner, since I left Minnesota, Dr. Colle has measured urinary concentration of corticosteroids in the postoperative newborn. She has found them to be low, as they are in normal newborns during the first week of life. When, in the second week of life, she treated an infant who had undergone surgery in the first week of life with ACTH, there was a good corticoid response. She has also done some preliminary studies on aldosterone levels in these infants and has found none. Dr. McCrory, we are also puzzled by the inability of Infant No. 16 to retain potassium and to correct its metabolic alkalosis as long as it was receiving intravenous fluid and by its rapid correction after oral feedings were introduced. Unfortunately, aldosterone levels were not run on these urines, but this can still be done. It would be interesting to know whether or not newborns can respond with an increased aldosterone production when stimulated properly.

34. "Vitamin D Activity" in Idiopathic Hypercalcemia

FRANCIS X. FELLERS * and ROBERT SCHWARTZ, Harvard

Two infants with the severe type of hypercalcemia (Fanconi-Schlesinger) characterized by mental and physical retardation and marked osteosclerosis were observed for 22 and 36 months. Studies related to calcium metabolism were carried out and included (1) balance measurements, (2) renal response to acute phosphate loading, and (3) measurement of vitamin D activity in serum.

Removal of all possible exogenous sources of vitamin D alone from an otherwise normal diet for six months failed to decrease the hypercalciuria or the hypercalcemia in either child. Addition of sodium phytate to the normal diet resulted in no change.

When both infants were fed a synthetic diet (15 mm. per day, calcium), calcium absorption was unusually high, varying from 36%-42% and 14%-24% of intake when growth was minimal. Removal of calcium intake (less than 1 mm. per day) from the diet resulted in marked positive balance of magnesium for the next five day period. Calcium and phosphorus balance were persistently negative and in proportions found in bone. In both infants, although hypercalciuria persisted, serum calcium returned to normal concentration. With resumption of growth, no radiological or biochemical evidence of rickets was observed even though vitamin D and calcium were markedly restricted.

Renal function, as measured by inulin and PAH clerances, was depressed. In the first patient, phosphate loading resulted in a two fold increase in phosphate reabsorption when the serum phosphorus was raised from 4 to 7 mg/100 ml. serum.

The data suggested an endogenous vitamin-D-active substance in these patients. Therefore, weanling rats were made rachitic by the standard AOAC vitamin D method and fed serum aliquots mixed with ether-extracted skim milk powder. The line-test was performed at the end of the assay period. Validity of this bioassay was ascertained by positive results in sera of three normal infants on normal vitamin D supplements at the 2.0 ml. level (6/6 rats each) and at the 0.1 ml. level in sera from two children receiving massive doses of vitamin D by mouth, 100,000 units daily (3/3 rats each).

Serum of the first hypercalcemic patient showed vitamin-D-activity in .05 ml. (8/8 rats) and none at the 0.025 ml. level (0/2 rats) in a sample obtained at age of 12 months. This D-activity was persistent in serum samples at 19 months (3/3 rats) and at 27 months when the patient had received minimal exogenous vitamin D for 17 months (3/3 rats) at the same serum level of .05 ml. In the second patient, D-activity was found in the serum sample at age 30 months at a level of .05 ml. (5/8 rats).

These studies suggest that an essential feature of idiopathic hypercalcemia is a defect in the metabolism of vitamin D.

Discussion

DR. MELVIN GRUMBACH, New York: I would like to ask Dr. Fellers whether he has used cortisone in the management of these patients. If so, what effect did it have on the levels of vitamin D activity in the serum?

DR. HAROLD E. HARRISON, Baltimore: This study, of course, fits in with one of the theories that has been suggested for the pathogenesis of hypercalcemia, namely, that there is not an excess of vitamin D due to increased intake of vitamin D, but rather some abnormality in the degradation or detoxification of vitamin D. The only other studies, as yet unpublished, which I know of along these lines were done by Morgan of Scotland. He determined vitamin D levels in two patients with "idiopathic hypercalcemia." I regret that I do not have any more details. He did not find elevated levels of vitamin D in the plasma by the bioassay technique. Would there be some way of reconciling the results of Dr. Morgan's study with the present report? Dr. Warkany is here, and I wish he

* By Invitation

would comment on the levels of vitamin D which he obtained in patients given massive doses of vitamin D. As I recall it, these levels reported today are not as large as those which he found in his patients. If I am mistaken on this point, I wish Dr. Warkany would correct me.

Dr. Heinz Berendes, Minneapolis: I have had a similar question about the effect of cortisone in these children. We have had the opportunity to see three patients with idiopathic hypercalcemia. One was followed for a period of over five years, the second one, for about 26 months, and the third child, for about one year. Similar balance studies as mentioned here were carried out with approximately the same results. One of the children representing a classical example of the severe form of idiopathic hypercalcemia was tried on a very low calcium intake without vitamin D for a period of three months resulting in only a minimal drop in serum calcium levels. No improvement in clinical symptomatology was observed. This patient was then placed on 30 mg. of cortisone daily with marked improvement in clinical symptomatology and return of calcium levels to normal in one month. Cortisone was then reduced to 10 mg. daily for another two month period. Subsequently cortisone was discontinued, and calcium intake was raised to 700 mg. daily, and 1000 units of vitamin D were added daily for a two month period. No return in symptomatology occurred. If the defect in vitamin D activity as described by Fellers and Schwartz accounts for the manifestation of idiopathic hypercalcemia, it would be a transient one in the benign form of hypercalcemia, and it seems to be rendered permanently reversible by cortisone therapy even in the severe form of this disease.

Dr. Charles U. Lowe, Buffalo: I wonder whether the authors made any attempts to keep their patients out of the sun during the period when they estimated the level of vitamin D.

Dr. Paul Gyorgi, Philadelphia: Apparently Baltimore is a big city. Dr. Morgan, as part of the team of Dr. John Howard, presented a paper yesterday at the Association, and he reported results with analysis of vitamin D in idiopathic hypercalcium indicating low vitamin D levels in serum. The only difference between the report presented here and that presented yesterday up at Haddon Hall is that the cases of Dr. Morgan were not malignant types, while, if I am correct, your cases were of the malignant group. Still, of course, I cannot understand why in the ordinary idiopathic hypercalcemia which everybody related to high vitamin D intake the serum vitamin D levels were low.

Dr. Jack Metcoff, Chicago: I wonder if Dr. Fellers will tell us if any studies have been done on calcium-binding by proteins during severe idiopathic hypercalcemia.

Dr. Malcolm A. Holliday, Pittsburgh: There was a recent report that sulfate would, in forming undissociated calcium, greatly enhance the excretion of calcium. Would sulfate administration be of value here both in providing more information about calcium metabolism and perhaps in lowering serum calcium in these children?

Dr. Robert M. Blizzard, Columbus, Ohio: Dr. David Smith, now at Madison, Wisc., formerly of Hopkins, has had an opportunity to study such a patient with idiopathic hypercalcemia. Vitamin-D assay was done on this patient and found to be elevated. This vitamin-D elevation continued over a year period, slowly falling toward normal as the serum calcium fell.

Dr. Francis X. Fellers: First of all, we did not give cortisone to any of these infants. I think that in England it has been given very frequently and certaintly results in improvement clinically, although continued administration might not show continuing improvement. Perhaps it would be dangerous in that it would further increase these infants' susceptibility to infection. We have differentiated these particular children from the so-called mild form and felt that the more prolonged sort of dietary therapy was indicated. In reference to the calcium-binding question, we did do a spinal fluid calcium test, but a simultaneous serum calcium was normal. It has been reported from England that the amount of calcium-binding is approximately normal with these children who have normal serum proteins and that the increase in the calcium is due to the ultrafilterable calcium itself. In relation to infusing sulfate, actually all three children have increased calcium excretion in the urine. In the face of marked reduced renal function, I do not think this would be a good form of therapy. As to the localization of this D-activity in the serum, yes, we have carried through with some of these studies. In two of the children, we have demonstrated by ultracentrifuge technique and separation of the serum into the lipoprotein fractions that there is approximately two to four times as much of the D-activity in the low density S-F10-40,000 β-lipoproteins as in the group S-F3-9 or α-lipoprotein fractions. This is in the same globulins that were mentioned yesterday in the talk by Dr. Howard. The serum D-activity from an adult who, I believe, was given large doses of vitamin D did show up in the α- 1 and 2 globulins.

35. Methemoglobin Reduction in the Erythrocytes of Newborns: Further Evidence of "Enzymatic Immaturity" in the Newborn

Jean P. Dawson* and Jane F. Desforges,*
Tufts.

Introduced by Sydney S. Gellis.

There has been increasing evidence in recent years of enzymatic immaturity in the newborn.

* By Invitation

It is the purpose of this paper to report studies revealing deficient methemoglobin reduction by the erythrocytes of newborns.

Cord blood erythrocytes were exposed to sodium nitrite in order to produce methemoglobin, and serial measurements of the methemoglobin were made after suspension of the cells in various substrates. Eighteen cord blo. ⁱs and 20 normal adult bloods were studied. The mean reduction of methemoglobin in the presence of glucose, lactate, or lactate and methylene blue was significantly less than normal after 300 minutes incubation, and in three cases the reduction was minimal, resembling the picture seen with congenital methemoglobinemia. In 10 cases reduction was similar to that seen in normal adults.

The reduction of methemoglobin in the presence of glucose and methylene blue was normal in all but two cases, both of whom were glutathione unstable, one with deficient glucose-6-phosphate dehydrogenase.

It has been suggested previously that the in vivo reduction of methemoglobin is normally mediated through a $DPNH_2$-dependent methemoglobin reductase, the $DPNH_2$ being generated upon oxidation of triose phosphate to glyceraldehyde phosphate, products of glucose breakdown. This $DPNH_2$-dependent reduction can also be produced in vitro by the oxidation of lactate to pyruvate upon the addition of the former as a substrate. A second incompletely represented pathway of methemoglobin reduction is that mediated by a $TPNH_2$-dependent methemoglobin reductase which acts only in the presence of glucose and methylene blue or similar substances. In this case $TPNH_2$ may be generated by the oxidation of glucose-6-phosphate to 6-phosphogluconate or of 6-phosphogluconate to pentose phosphate in the presence of their respective dehydrogenases. Lonn and Motulsky have previously shown a deficiency of $TPNH_2$-dependent methemoglobin reductase in cord bloods.

It is apparent from our studies that in most newborns, although $TPNH_2$-dependent methemoglobin reduction is normal, in occasional infants it may be deficient due either to decreased methemoglobin reductase or decreased $TPNH_2$ generation. On the other hand, many infants appear to have some deficiency of $DPNH_2$-dependent methemoglobin reduction and an occasional case appears to have a severe defect. This is possible due to either a deficiency in methemoglobin reductase or in $DPNH_2$ generation.

The frequency of deficient $DPNH_2$-dependent methemoglobin reduction in newborns may explain the apparent increased susceptibility of infants to oxidizing agents such as nitrate-containing well water, aniline, and benzocain.

Discussion

DR. BENJAMIN M. KAGAN, Los Angeles: I wonder if the authors could tell us whether they have some information which would explain a curious finding reported a few years ago (A. M. A. J. Dis. Child., 91:1-5, 1956). We found that premature infants after 24 hours of age and up to 7 days of age had significantly higher blood methemoglobin levels than those younger or older and higher than full term infants or older children. I wonder if studies such as you are doing now might explain these observations.

DR. WILLIAM KRIVIT, Minneapolis: As a corollary to the "enzymatic immaturity" of the erythrocytes of the newborn, I have observed that the autogenous red cell survival of newborns is markedly shortened. The survival study was done by using chromium; objections to this method of "elution" or added reduction of the already immature red cell cannot account for the increased rate of destruction noted by Cr^{51} survival method. The T 1/2 obtained is from 12-15 days, or approximately twice that of normal adults. I would wonder if the author would like to speculate as to the relationship between the reduced enzymes present in newborn red cells and the hyperbilirubinemia of the newborn?

DR. J. LUCEY, Burlington, Vt.: I would like to ask Dr. Dawson whether she has any feelings as to the possible role of this enzyme deficiency in the apparent sensitivity of premature infants to large doses of vitamin K.

DR. JEAN P. DAWSON: Dr. Kagan, regarding the hemoglobin level in the premature infants, we have studied some premature infants and have done methemoglobin levels, particularly in relationship to the administration of vitamin K, and we have found no significant high level during the course of these studies, which I believe extend up to about the age of one week. In regard to Dr. Kravit's comments about the life span of the red cells in the newborn period and the relationship to reduction of enzyme activity, we have no information on the correlation between enzyme levels and life span of erythrocytes. There have been statements in the literature that high methemoglobin levels lead to hemolysis but such experiments have involved the use of drugs that may affect red cell membrane integrity. Perhaps this defective mechanism may be working in such cases. I really do not know. We have studied other enzymes that may have some bearing on this problem. Significantly, the one we have found lowered in our infants has been glutathione reductase. The 6-phosphate dehydrogenase is elevated. Whether the glutathione reduction is related to hemolysis and to a shortened life span of newborn erythrocytes, we do not know at present. The relationship between vitamin K and

premature infants, again, relates to our study which is actually a study with Dr. Gellis and Dr. Gloria Jeliu.

36. Intracardiac Phonocardiography in the Diagnosis of Congenital Heart Disease

DAVID H. LEWIS*; ALI ERTUGRUL*; GEORGE W. DEITZ*; JOHN D. WALLACE*; JAMES R. BROWN JR.,* and ABDOL-NABI MOGHADAM,*
Philadelphia General Hospital, and the U. S. Naval Air Development Center, Johnsville, Pa.
Introduced by LEWIS A. BARNESS.

During the past two years studies have been carried out on the determination of heart sounds from within the heart in patients with a wide variety of cardiovascular disorders. The purpose of this communication is to present the results in the first 57 patients with congenital heart disease. Of this group 51 ranged in age from 1 week to 13 years. Six of the patients with congenital heart disease were between the ages of 16 and 40. In addition to this, patients have been studied who were suspected of having heart disease and who were found subsequently not to have heart disease. Of this group, 10 were in the age range of 1 to 13 years.

The technique used is an adaptation of that developed by the United States Navy for undersea warfare. It involves the passage of a specially designed cardiac catheter which has at its tip an underwater microphone. After suitable amplification, the sounds are recorded, using a multichannel photographic oscillograph, on magnetic tape. This phonocardiographic technique is able to localize the production of heart sounds and murmurs to an extent not heretofore obtainable, and it is this property which makes it of particular value in the diagnosis of congenital heart disease.

In patent ductus arteriosus there is a machinery murmur which is loudest in the pulmonary artery in the region of the ductus. It is not heard in the aorta, but begins within the ductus itself and becomes full blown in the pulmonary artery at the entrance of the ductus. It may be localized to this area, or if the ductus is large it may be present throughout the pulmonary vascular tree, but it is not present in the ventricle. In pulmonic stenosis the characteristic systolic murmur is heard best in the main pulmonary artery but is not heard in the ventricle. This same murmur has been heard in the pulmonary veins and in the left atrium. In ventricular septal defect the characteristic systolic murmur is loudest in the ventricle and it may be localized to the region of the defect but it is not present in the atrium. With the combination of pulmonic stenosis and ventricular septal defect, the characteristic systolic murmurs are present at the respective locations (i. e., the murmur of pulmonary stenosis is heard in the pulmonary artery, and the murmur of ventricular septal defect is heard in the ventricle.) In atrial septal defect there is a systolic murmur in the pulmonary artery which closely resembles that heard on the chest wall at the mid or upper portion of the sternum. A diastolic murmur beginning with the opening of the tricuspid valve has been observed in the inflow tract of the right ventricle which closely resembles that heard on the chest wall at the lower portion of the sternum. There is also a different diastolic murmur heard in the right atrium. In aortic stenosis the characteristic systolic murmur is heard throughout the thoracic aorta, but is not heard, for example, in the pulmonary artery. In coarctation of the aorta a systolic murmur is present in the ascending aorta, and in the aorta in the region of the coarctation there is a systolic and diastolic murmur.

Lantern slides of these lesions will be presented along with a discussion of the method. Tape recordings of several lesions will be played if time permits.

By localizing the site of murmur and sound production, intracardiac phonocardiography has proved a valuable addition to the studies used to establish an exact diagnosis in patients having, or suspected of having, congenital heart disease.

Discussion

DR. K. WOODWARD, Rochester, N. Y.: Am I correct that the patient would have been three weeks of age? If so, could you tell us what the possible oscillatory findings on the chest might be?

DR. DAVID H. LEWIS: In the slide that I showed of the girl one week old—there were two, one was one week old, the other was three and one-half years old—there was a loud harsh systolic murmur heard on the chest. No diastolic murmur was heard. I think it is fair to say that most of the clinicians did not suspect the patent ductus arteriosus to be the diagnosis here.

37. Studies of Normal and Malignant Cells in Tissue Culture: The Effects of Heterologous Immune Serums upon HeLa Cells in Vitro and Rat-HeLa Tumors in Vivo

ROBERT M. MCALLISTER; PAUL G. GRUNMEIER,* and LEWIS L. CORIELL, Pennsylvania, and The South Jersey Medical Research Foundation, Camden, N. J.

The human tissue culture cell lines, HeLa, was used as a cancer cell antigen for the production of heterologous immune serums in rabbits, rats and a cow. The effects of these immune serums upon HeLa cells were studied *in vitro* by means of a

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metabolic inhibition test and *in vivo* in the rat-HeLa tumor technique.

Both tests made possible a high degree of quantitation and reproducibility not previously available in the study of human cancer.

The *in vitro* metabolic inhibition test recently described by Salk was found to be easily quantitated and reproducible. The cytotoxic activity of the cow immune serum was heat resistant, complement dependent, gradually increased in amount during immunization of the cow and was not found in normal serums of any species tested.

The rat-HeLa tumor technic was used to study the effects of these immune serums *in vivo*. X-irradiated and cortisone-treated weanling rats were inoculated intraperitoneally with various volumes of immune serums and inoculated the following day with HeLa cells.

The volume of immune serum per cell which prevented rat-HeLa tumors *in vivo* correlated closely with the volume of immune serum which prevented growth of HeLa cells *in vitro* in the metabolic inhibition test.

Discussion

DR. RICHARD T. SMITH, Dallas, Texas: It was a pleasure to hear about this beautiful technique. I would like to ask a question, however. The calculations described a space occupied by the antibody as about 60% of body weight. I would like to know a little more about the method of calculating the space, because most heterologous proteins occupy a space in adult animals not in excess of 10% of the weight in any animal which we have worked with so far.

DR. ROBERT M. MCALLISTER: Thank you, Dr. Smith for your comment. I do not have the data with me, but I will take a swing at it as follows: 0.1 ml. of the immune cow serum should kill 160,000 cells according to in vitro cytotoxic test. A 1 to 16 dilution should kill 10,000 cells. When we tested 0.1 ml., we obtained no activity. Therefore, we went back and tested 0.25 ml. in which, on the basis of our predicted killing, one would expect 25,000 cells to be killed. Actually, the test indicated 40,000 cells killed. Therefore, we expressed this as the estimate that the immune serum injected went into 60% of the rat's body weight.

38. Growth of Human Cancer Cells on In Vivo Tissue Culture

DONALD PINKEL* and G. DAVID BAER,*
Roswell Park Memorial Institute, and
University of Buffalo
Introduced by MITCHELL I. RUBIN.

Although many types of human tumors have been successfully transplanted to hamsters and rats conditioned with cortisone and radiation (Toolan, Cancer Res. 14:660, 1954), attempts to grow leukemia and lymphoma have failed. In the present study it was found possible to grow human leukemic and certain other cancer cells in hamsters using an in vivo tissue culture technique.

Algire et al. (J. Nat. Cancer Inst. 15:493, 1954) described a method of in vivo tissue culture, utilizing chambers constructed from plastic rings and millipore plastic membranes which are implanted in the peritoneal cavity or soft tissue of experimental animals. The membrane is intended to allow diffusion of gases, metabolites, and proteins but to prevent contact of host cells with the cultured tissue. Homografts within such chambers do not initiate immunity, and they are not affected by previously induced host immunity, but the situation regarding heterografts is not clear (Weaver et al., J. Nat. Cancer Inst. 15:1737, 1955; Algire, Ann. N. Y. Acad. Sc. 69:663, 1957). Using this method, ovarian homografts have been kept alive and functioning for long periods in the rat (Sturgis and Castellanos, Proc. Soc. Exper. Biol. & Med. 94:569, 1957), and growth and division of HeLa cells in mice have been observed for limited periods (Algire et al., Proc. Am. A. Cancer Res. 2:90, 1956).

The buffy coat layers of peripheral blood and bone marrow of patients with acute leukemia in relapse were placed in diffusion chambers which, after intraperitoneal implantation in postweanling hamsters, were removed after 10, 20, and 30 days. At 10 days all samples showed necrosis with relatively few viable cells, but at 20 days leukemic cells were found to be growing and dividing as evidenced by increase in cell population and the presence of mitotic figures. These cells bore close similarity to the original cells, which is in contrast to what has been observed in in vitro tissue cultures of leukemic cells (Osgood, in "The Leukemias: Etiology, Pathophysiology and Treatment," edited by Rebuck, Bethell and Monto, New York, Academic Press, 1957, pp. 227). In many cases, however, only monocytoid and giant cells or no viable appearing cells at all were recovered at 20 and 30 days, and to date no dividing leukemic cells have been noted at 30 days.

Fragments of a Ewing's sarcoma implanted in a similar way were found to have viable and actively dividing sarcoma cells surrounding a necrotic center at 10 days. At 20 and 30 days this peripheral portion appeared to be thinner and to consist of mature fibrous tissue. Nerroblastoma cells appeared to show differentiating characteristics at 10, 20, and 30 days, besides the necrosis in the first 10 days, a finding similar to observations made on in vitro tissue culture of this tumor (Murray and Stout, Am. J. Path. 23:429, 1947). A strain of rabbit

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bone marrow explanted from continuous in vitro tissue culture was observed to grow and actively divide for up to 30 days under the conditions of this experiment.

Empty diffusion chambers were implanted intraperitoneally in hamsters and the clear liquid contents were electrophoretically analyzed after 10 days for serum proteins. The globulin fractions were present but in significantly lower concentrations than in the hamster serum. Smears of this fluid were consistently negative for host cells.

The growth of human leukemic cells in a heterologous host indicates the capacity of such cells to grow autonomously and may be interpreted as evidence that acute leukemia, at least in some instances, is a true neoplastic disease and not a tissue alteration dependent on environmental factors within the patient.

This technique of heterologous growth of human leukemia cells may be useful in the study of the disease and in the screening of potential antileukemic agents. Methods of achieving more consistent growth over longer time periods are being studied.

Discussion

DR. RICHARD T. SMITH, Dallas, Texas: I would like to be sure I understood correctly the circumstances of the 30 day necrosis of the cells in the Algire chamber. Was this seen with isologous or autologous cells? The interpretation of the fate of the explanted tissue depends heavily on the origin of these cells.

DR. DONALD PINKEL: When diffusion chambers containing isologous tumor cells are implanted in mice, an initial necrosis occurs just the same as when homologous or heterologous cells are used. Within a few days the cell population begins to increase, usually reaching a peak in 10 to 30 days (Amos and Wakefield, J. Nat. Canc. Inst., to be published). As mentioned before, homologous tissues may also be maintained for long periods in diffusion chambers but heterologous material becomes necrotic by 25 or 30 days. Some studies have been made of immunologic relations between chamber contents and host. We have implanted chambers containing Type A Rh positive human red blood cells in hamsters and have been unable to detect hemagglutinating properties against these red cells in the animal serum after 10 or 15 days. On the other hand, subcutaneous injection of the same red blood cells does lead to development of hemagglutinins in the animal serum, apparently of the incomplete antibody type since addition of albumin is required for their demonstration.

39. The Seromucoid Values in Hypothyroidism and Hyperthyroidism as Determined by a New Technique

S. HARSHMAN*; J. B. SIDBURY JR., and W. H. PRICE,*
Johns Hopkins Hospital

Variable results have been obtained in the seromucoid levels of patients with hypothyroidism and hyperthyroidism, but in general the values have been low in the former and high in the latter. It has been clearly demonstrated that there is a deposition of glycoprotein in the skin and subcutaneous tissue of patients with myxedema, indicating an extensive abnormality of polysaccharide metabolism.

A method has been developed for the partition of seromucoid with Dowex 50 ion-exchange resin. The serum of normal individuals reveals three characteristic peaks. Nine of eleven hypothyroid patients showed a peak coming off the column earlier than the first normal peak (Tube 25). Five of seven patients with hyperthyroidism and one with simple goiter showed an abnormal peak (Tube 186) coming off the column much later than the third normal peak. Two hyperthyroid patients had no early peak, but showed a peak in Tube 186. Three hyperthyroid patients showed the abnormal early peak, one of these subsequently had a 186 peak.

The particular seromucoid pattern obtained in a patient with thyroid dysfunction does not reflect the physiologic status of the patient. Treatment does not appear to affect the pattern. No other disease surveyed thus far had either a 25 or a 186 peak.

Discussion

DR. ROBERT M. BLIZZARD, Columbus, Ohio: I would like to ask Dr. Sidbury what the significance of the two peaks may be and how this might be related to TSH, for example.

DR. PARKS S. GERALD, Boston: Have you done any family studies of the patients who have these abnormal peaks?

DR. JAMES B. SIDBURY JR.: These are both very good suggestions. We plan to collect, pool, and assay the material from peaks 25 and 185 for TSH activity. We have collected sera from parents of some of these patients and plan to run them. I feel too that this might be profitable.

Papers Read in Special Sessions

40. Pulmonary Valvular Stenosis with Intact Ventricular Septum in Infants

MARY JANE LUKE*; CATHERINE A. NEILL,* and HELEN B. TAUSSIG, Johns Hopkins

The syndrome of valvular pulmonary stenosis with an intact ventricular septum in infants has

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received relatively little emphasis. It is, however, extremely important because the condition may cause death in infancy and furthermore, the difficulty can be relieved by operation. The present report emphasizes the indications for early surgery in this usually benign condition and the urgency of pulmonary valvulotomy once signs of heart failure have appeared.

A large number of infants with this diagnosis have been seen but we discuss here only the 26 patients who underwent either surgery or autopsy between the ages of two months and two years. The clinical picture was characterized by a harsh systolic murmur heard in all patients shortly after birth, and in 14 of the 26, cyanosis also dated from under one month of age. In the early weeks, the infants gained well and appeared sturdy. The earliest symptoms of cardiac difficulty were usually restlessness and irritability, often accompanied by a slowing of weight gain. Motor development, previously normal, often ceased as in one baby who sat up at five months but thereafter made no progress and on admission at 17 months was still not crawling. The retardation of weight gain and motor development was accompanied by appearance or deepening of cyanosis. Eight infants had "cyanotic spells" consisting of rapid respirations, limpness, and transient unconsciousness; four infants died during such episodes which are of grave import in this condition and warrant immediate surgery. In all but one infant, the spells were accompanied by signs of congestive failure. The onset of severe right heart failure was often heralded by lethargy and a weak cry, followed by edema, frequently well seen in the face. Edema was a late and ominous sign but had, in two instances, been attributed elsewhere to nephrosis and in other instances, had been treated for several weeks with digitalis and mercurials.

Physical examination revealed a systolic murmur in all cases; in most infants the murmur was harsh, stenotic in character, and maximal in the pulmonary area, but in a few was best heard at the lower left sternal edge. One patient with an associated patent ductus also had a continuous murmur. In some of the most severe cases, the murmur was soft and had decreased in intensity with the onset of cardiac failure. The pulmonary second sound was diminished or absent. Cyanosis of varying degrees was always present and was accompanied by polycythemia in 21 patients. Signs of right-sided cardiac failure, dyspnea, hepatomegaly, and edema were combined in many with presystolic hepatic pulsations and giant *A* waves.

X-ray showed a characteristic globular heart with a prominent right atrium and ventricle and clear lung fields. Poststenotic dilatation of the pulmonary artery was slight or absent.

The electrocardiogram showed severe right ventricular hypertrophy with ST depression and "strain pattern"; in several patients progressive electrocardiographic changes indicated impending cardiac enlargement and failure. The tall peaked P waves of right atrial hypertrophy were consistently present.

Cardiac catheterization was undertaken in seven patients, and it revealed a systolic gradient across the pulmonary valve of between 100 and 200 mm. of mercury.

Six infants died before surgery could be undertaken; in four, death was in a cyanotic spell; the two others were in extremis, and we erred in attempting to improve their condition medically prior to surgery. Twenty infants underwent surgery by the transventricular approach; in 17 a good result was obtained with dramatic clinical improvement and rather slower improvement in heart size and electrocardiogram. These patients have been followed for between six months and five years and have remained well. Three patients died at or immediately after surgery; all were in advanced failure, and one had undergone a previous anastomosis. In both surgical and autopsied cases, the valve was domeshaped and frequently pinpoint in size.

Infants with this diagnosis should be followed at three-to-six-month intervals during the first two years of life and surgery should be considered for increasing cyanosis, cardiac enlargement or electrocardiographic deterioration. Surgery is urgently indicated if cardiac failure or cyanotic spells develop, and digitalis and diuretics should be used only as ancillary aids. A good result was obtained in 17 of the 20 infants submitted to surgery.

41. The Effect of Penicillin on Acute Rheumatic Fever and Valvular Heart Disease

EDWARD A. MORTIMER JR.*; SAMUEL VAISMAN*; AUGUSTO SCHUSTER*; JULIO GUASCH*; ALBERTO VIGNAU*; LOUIS RAKITA*; CHARLES H. RAMMELKAMP JR.,* and RICHARD KRAUSE,*
Western Reserve and the University of Chile
Introduced by FREDERICK C. ROBBINS.

Although the acute symptoms of rheumatic fever are readily controlled by several different drugs, no form of therapy has been proved to exert a marked effect on acute carditis or the incidence of subsequent valvular heart disease. The importance of the streptococcus in the initiation and development of acute rheumatic fever suggested that the elimination of this organism at the time that the signs of rheumatic activity were manifested might alter either the acute phase symptoms or the incidence of valvular heart disease or both.

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Accordingly, a controlled study was undertaken in which massive doses of penicillin were employed to eliminate the streptococcus from patients with acute rheumatic fever. Forty-nine patients received penicillin for six weeks, and 48 received no antibiotic and served as controls. After the initial six weeks of hospitalization, both groups were placed on benzathine penicillin prophylaxis for one year.

Evaluations of the cardiac status were made daily during the initial six weeks and at one year by an observer who was unaware of the treatment administered. Acute phase phenomena were observed at frequent intervals.

The results demonstrate no significant differences between the two groups in symptomatology, laboratory phenomena, or cardiac manifestations during the first six weeks. However, at the end of one year a difference of highly probable statistical significance was noted in the incidence of organic murmurs in those patients without advanced valvular heart disease on admission. The results would indicate that the administration of penicillin may be the preferred method for the treatment of valvulitis, but such therapy should be supplemented by other drugs to control the acute phase symptoms.

42. Phonocardiographic Characteristics of Atrial Septal Defect

ROBERT EISENBERG * and HERBERT HULTGREN,*
Stanford
Introduced by JOHN OSBORN.

Since atrial septal defects of the secundum variety can now be safely closed by surgery, correct preoperative diagnosis becomes important. This lesion is associated with a large left to right shunt and a delay in electrical depolarization of the right ventricle. Both these features may produce distinctive changes in the heart sounds. Phonocardiography therefore may be an important technique in the correct identification of patients with such lesions. It is the purpose of this paper to describe the phonocardiographic characteristics of 20 patients (aged 5 to 50 years) with atrial septal defects whose diagnoses have been established by cardiac catheterization. All had mean pulmonary artery pressures below 35 mm. Hg and calculated left to right shunts of from 2.3 to 11.3 L/min/square meter (mean, 5.6 L/min/square meter). The QRS duration varied from 0.07 to 0.11 second (mean, 0.091 second). Ten patients had surgical closure of the defect, and heart sounds were studied before and after surgery.

Phonocardiograms were recorded with a Sanborn Twin Beam Cardiette at rest and after a short period of vigorous exercise. Sounds and murmurs were correlated with simultaneously recorded electrocardiograms, carotid and apex impulse tracings, as well as intracardiac pressure curves. The following observations were made:

A systolic ejection murmur was recorded at the pulmonic area in all patients. The murmur was loudest just prior to the middle of systole and its intensity was roughly proportional to the size of the left to right shunt. Following surgery the murmur disappeared (three patients) or became very faint (seven patients). Splitting of the second sound was present in all patients, pulmonic valve closure following aortic valve closure by 0.05 to 0.09 second (mean, 0.063 second) in expiration compared to the normal value of 0.01 to 0.05 second (mean, 0.023 second). The degree of splitting decreased slightly following surgery (mean decrease, 0.02 second). Splitting of the first heart sound at the lower left sternal margin or apex was present in 17 patients. Mitral closure preceded tricuspid closure by 0.02 to 0.07 second (mean, 0.04 second) compared to the normal range of 0.01 to 0.04 second (mean, 0.026 second). No change in these values occurred as a result of surgery. In six patients adequate phonocardiograms were obtained after exercise. In each instance the intensity of the heart sounds and the systolic murmur was increased. Splitting of the second heart sound decreased by 0.02 to 0.03 second. Exercise produced similar changes following closure of the septal defect.

The following phonocardiographic features appear to be characteristic of operable atrial septal defects.

1. Splitting of the second sound at the pulmonic area. The degree of splitting decreases but does not disappear following surgery and therefore may in part be related to the increased right ventricular output.

2. Splitting of the apical first sound which is not affected by surgery and may be due to the delayed onset of right ventricular contraction.

3. A systolic murmur at the pulmonic area which is probably produced by flow across the pulmonic valve since the intensity of the murmur is related to the magnitude of the pulmonary blood flow.

43. Basic Hemodynamics in Infants and Children

ABRAHAM M. RUDOLPH *; MILTON H. PAUL *; GLENN C. CAYLER,* and JULIEN I. E. HOFFMAN,*
Harvard
Introduced by ALEXANDER S. NADAS.

Cardiac catheterization studies for evaluation of heart disease in infants and children have been performed by numerous investigators. However, only limited information is available pertaining to basic dynamics of the circulation in the younger age group. The present report is based on cardiac catheterization studies performed without general

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anesthesia, in over 750 individuals of whom 20% were under one year of age.

Analysis of oxygen saturation data in infants and children showed that, as in adults, there is little variation between saturations in venae cavae, right atrium, right ventricle, and pulmonary artery. However, a gradual decrease in mixed venous saturation during a long period of study (one to two hours) has been demonstrated. The importance of assessing oxygen saturation rather than oxygen content data in evaluating shunts in infants will be discussed.

Arterial oxygen saturation is frequently reduced in infants. Pulmonary venous saturation measurements have demonstrated that in many instances this is due to a pulmonary factor rather than to right-to-left shunt. The significance of this information in calculation of pulmonary blood flow and estimating shunts is presented.

Cardiac output was calculated by the direct Fick method. Oxygen consumption in the infant age group was measured by the open circuit method with the aid of a special mask. A good correlation between resting cardiac output and body surface area, already established in adults, was demonstrated in all age groups. The derived regression lines indicated that cardiac output in the infant age group was very close to adult figures based on body surface area. The validity of expressing vascular resistance in relation to body surface area for comparison of various age groups is discussed on the basis of this information.

44. Studies on the Type-Specific Immune Response to Group A Streptococcal Infections in Children

ALAN C. SIEGEL and GENE H. STOLLERMAN, Northwestern

The only antibody known to confer immunity against group A streptococcal infection is that which is directed against the type-specific M protein antigen of these organisms. Methods for the detection of this antibody are difficult, and information about its behavior in childhood infection is relatively scarce. The only extensive studies of this antibody have been made in epidemics occurring in military populations. A large scale controlled study of group A streptococcal infections in a pediatric outpatient group is in progress and has afforded the opportunity to study the development and persistence of type specific antibody in patients with uncomplicated untreated streptococcal pharyngitis and the effect of penicillin therapy upon its formation.

Ninety-eight children with infections of known serologic type were selected for study. These included 72 children with type 12, and 26 children with type 3 group A streptococcal infections who were followed for 6 to 18 months from the time of onset of the acute pharyngitis. Serum samples were obtained on the 1st, 21st, 35th, and 60th days following the infection and at six-month intervals thereafter. One half of the children were treated initially with 600,000 units of benzathine penicillin intramuscularly, and the other half were treated symptomatically.

Bactericidal tests for the measurement of type specific antibody were performed on all sera using Lancefield's recent refinement of Todd's method. The same sera were also tested with another method for the detection of type specific antibody recently developed in our laboratories. The latter depends upon the phenomenon of long chain formation of certain strains of group A streptococci when grown in liquid media to which homologous type antisera have been added.

Of 48 children with untreated infections due to either type 12 or to type 3 organisms, 60% developed measurable type specific antibody compared with 10.5% of the 48 children with penicillin treated infections.

The most striking difference in the behavior of anti-M antibody, compared with antistreptolysin O, antihyaluronidase and antistreptokinase titers was the relatively long delay in its appearance. It was usually not detected before the 60th day, or longer, from the onset of infection in contrast to the other antibodies which showed rises in titer by the 21st or 35th day. This finding is in agreement with other studies.

The frequency of development, and the persistence of anti-M antibody was related to the duration of the convalescent carrier state. When group A streptococci were eradicated from patients' throats by penicillin therapy, or when they spontaneously disappeared from the throat of the untreated patient early in convalescence, anti-M antibody rarely developed. Conversely, those who carried the organism in their throats for several weeks or more developed anti-M antibody most frequently and persistently.

The results indicate that treatment with a penicillin regimen that effectively eradicates the organism also results in striking suppression of acquired type specific streptococcal immunity. The percentage of untreated patients who developed measurable antibody to M protein is considerably less than that reported in epidemic streptococcal infections in military recruits. This may indicate a difference in the nature of sporadic streptococcal infections in children.

45. Effect of Acetylcholine on Pulmonary Hypertension in Patients with Ventricular Septal Defects

I. HUNTER CRITTENDEN * and FORREST H. ADAMS, University of California, Los Angeles

Vascular changes associated with pulmonary hypertension in left-to-right cardiac shunts have

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been shown to be an important factor in operability of such patients. Pulmonary resistance to flow is effected by vascular alterations in terms of medial hypertrophy and later intimal proliferation and hyalinization. Medial hypertrophy while increasing pulmonary resistance is capable of vasorelaxation and hence is theoretically reversible. Intimal changes on the other hand produce marked resistance to flow and may fix the vascular bed in a rigid state incapable of vasomotor action. The operative risk has been correlated with the severity of such vascular disease.

Many patients have been operated upon and have not survived surgery, because the exact state of their diseased vessels was not known preoperatively.

The present study was undertaken in an effort to determine the flexibility of the pulmonary vascular bed by means of a cholinergic drug as an index of vascular change and of operative risk.

Acetylcholine has a known action on tissues influenced by autonomic function and has been shown to lower pulmonary artery blood pressure in pulmonary hypertension when due to vasoconstriction. Acetylcholine acts immediately at neuromuscular sites but is destroyed very rapidly in the blood stream. This allows immediate action on the pulmonary vessels when introduced into the main pulmonary artery, and because of its rapid destruction in passing through the lungs, systemic effects are not seen when moderate doses are used.

All of the patients investigated in this report were of pediatric age and had interventricular septal defects or patent ductus arteriosus with pulmonary hypertension as demonstrated by cardiac catheterization and angiocardiography. These studies were performed following the routine diagnostic catheterization under rectal basal anesthesia. Under basal conditions, a pressure tracing and blood oxygen saturation from the main pulmonary artery was obtained. Acetylcholine in doses from 2 to 15μg. per kilogram was rapidly injected into the main pulmonary artery and a continuous recording of the intracardiac pressures was made for the next several minutes. In some patients, blood oxygen samples were obtained at 30 seconds after injection to determine changes in left to right flow. Systemic effect was measured in some cases by means of a digit plethysmograph.

TABLE 1.—*Effect of Acetylcholine on Pulmonary Hypertension (IVSD Cases)*

Case	Age, Yr.	MPA Pressure Before	MPA Pressure After	Drop, %	L-R Shunt (of syst. flow), %
1	6	90/54	90/54	0	100 e *
2	9	85/74	85/75	0	33 before AC e 33 after AC
3	9	112/52	91/31	26	270 before AC 830 after AC
4	13	96/62	68/42	30	140 before AC 396 after AC
5	8/12	71/39	67/25	16	230
6	3	93/0	73/0(RV)	21	940 e

* e = Expired, surgical.

TABLE 2.—*Effect of Acetylcholine on Pulmonary Hypertension (Other Lesions than IVSD)*

Case	Age, Yr.	Diagnosis	MPA Pressure Before	MPA Pressure After	Drop, %	L-R Shunt (of syst. flow), %
1	2	A-V Com.	89/32	87/28	5	440
2	8	PDA VSD	100/36	100/38	0	40
3	3½	A-V Com.	53/25	47/17	18	264 e*
4	4	PDA	81/61	73/45	17	349 s†
5	9	PDA VSD	60/35	59/31	14	360

* e = Expired
† s = Survived surgery

Eleven cases of pulmonary hypertension due to left-to-right shunts have been studied with acetylcholine to determine the flexibility of the vascular bed. Four of these cases did not respond to the drug. Of these, two were operated on and the defects successfully closed but the patients later died from respiratory and right heart failure. Pulmonary blood vessels in both of these showed considerable intimal change.

The other seven cases responded to acetylcholine by a significant drop in pressure and, in those tested, an increase in left-to-right shunting. Three of these cases have had surgery, two expired from causes other than pulmonary malfunction. The blood vessels in these three cases showed medial thickening but no intimal change.

A consistent correlation was shown between the pulmonary vascular resistance and response to acetylcholine. Systemic effect was not observed in those cases measured.

The number of cases thus far studied is inadequate to draw definite conclusions. These preliminary results do suggest, however, that with more data, this test will be helpful in the selection of surgical candidates in those cases whose pulmonary resistance is a major consideration.

46. The Evolution of Metabolic and Ventilatory Changes in Respiration in the First Two to Five Weeks of Life

MILDRED STAHLMAN, M.D., Vanderbilt

Utilizing techniques previously described, the evolving metabolic and ventilatory aspects of the normal newborn infant's respiration have been studied serially during their first two to five weeks of life. The sleeping alveolar CO_2 tension in normal full-term newborns is 8 to 15 mm. Hg below normal adult levels (range, 25 to 33 mm. Hg). This tension closely parallels that of the mother just before delivery. The infant's tension rises gradually within two weeks to 36 to 40 mm. Hg whereas that of the mother rises more abruptly to about 40 mm. Hg.

The initial levels of O_2 uptake and CO_2 output are quite low for their surface area increasing as much as 100% during the first two weeks and more gradually thereafter. Minute ventilation and alveolar ventilation are also increasing remarkably, both continuing to rise more gradually after one week, with no marked changes from normal in physiological dead space.

When infants in their first three days breathed either 2% or 4% CO_2 up to 66 minutes, a steady state was never achieved as far as alveolar ventilation, CO_2 output, O_2 uptake, alveolar CO_2 tension, and dead space/tidal volume ratios were concerned. Realizing that a steady state would seldom if ever be achieved, a 25 minute equilibration period was chosen for serial weekly inhalations of 2% and 4% CO_2 during the first three to five weeks.

The newborn's response to increased inspired CO_2 tension fell roughly into two groups. The first response pattern was seen more regularly in infants in their first week of life. The second pattern was more common after the first week. The younger infants showed inadequate response to CO_2 with slight increases in minute ventilation and alveolar ventilation, and sometimes considerable decreases. Oxygen uptake was unchanged, but CO_2 output fell progressively on 2% and 4% CO_2. Alveolar CO_2 tension rose about 10 mm. Hg. By contrast, the same infants later in life often were able to increase minute ventilation by greatly increasing dead space breathing, so that alveolar ventilation was still inadequate, alveolar CO_2 tension still rose, and CO_2 output fell. Oxygen uptake was increased by the increased work of breathing.

The low alveolar CO_2 tension during pregnancy has been thought to be due to increased sensitivity of the mother's respiratory center to CO_2 related to high progesterone levels. Whether the low alveolar CO_2 tension in newborns is due to the placental transfer of progesterone cannot be stated with assurance, but we have been unable to produce with any consistency significant drops in CO_2 tension following progesterone administration to newborns. In addition, the newborn's response to CO_2 inhalation at a time when his CO_2 tension is quite low does not indicate any increase in sensitivity of the respiratory center. The great increases in dead space ventilation suggest abnormal ventilation/perfusion ratios. It is unlikely that the low initial alveolar CO_2 tension is due to low carbonic anhydrase levels since these persist longer than this change in CO_2 tension. It seems possible that the persistence of left-to-right fetal shunts with diminishing flows through the first week might account in part for the changes in CO_2 tension during this time.

47. Blood Oxygen Tension as a Measure of the Adequacy of Pulmonary Ventilation During the First Hour of Life

IRWIN WEISBROT,* Columbia
Introduced by RUSTIN MCINTOSH.

Many infants have an arterial blood oxygen saturation of 90% or less for an hour or more after birth. However, in the presence of a low pH and an elevated P_{CO_2} the O_2-hemoglobin dissociation curve is shifted to the right, and a low per cent saturation may represent a much higher oxygen tension than is expected.

Moreover, because the infant has a metabolic acidosis with CO_2 retention the level of P_{CO_2} is not an adequate guide to relative alveolar ventilation, nor can the Rahn and Fenn CO_2 Diagram be used at this age because of the nonbasal condition of the infant and the difficulty in collecting alveolar gas samples.

Therefore, the measurement of arterial blood O_2 tension is the most direct method of estimating the ability of the newborn infant to exchange respiratory gases with the atmosphere. A polarographic method for the measurement of blood O_2 tension has been developed. A series of determinations on cord blood and on arterial blood drawn at intervals during the first hour of life have been made. The results of these determinations will be discussed in relation to previous estimates of the rate of oxygenation after birth and in relation to the shape of the fetal O_2-hemoglobin dissociation curve at different pH and P_{CO_2}.

48. The Mechanism of Action of Phosphoglucomutase from Muscle and Yeast: Its Implication in Galactosemia

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The manner by which an enzyme performs its catalytic activity has been the subject of investiga-

* By Invitation

tion for some time. The only enzyme so far successfully studied from this standpoint is phosphoglucomutase. This is the enzyme that converts glucose 1-phosphate to glucose 6-phosphate. This enzyme is inhibited very markedly by galactose 1-phosphate, which can explain the damage that one observes in galactosemia. Both the muscle enzyme and the enzyme from yeast have the same mechanism of action. The enzyme molecule transfers a phosphate to the 6-carbon of glucose 1-phosphate to produce glucose 1-6-diphosphate. The latter then transfers the phosphate from the first carbon to the enzyme to produce glucose 6-phosphate.

This will be presented in an animated illustrative movie, showing the mechanism and the manner of inhibition by galactose 1-phosphate.

49. Observations on Glucuronic Acid Conjugation in Patients with Familial Nonhemolytic Jaundice and Their Families

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Bilirubin has been shown to be excreted as a glucuronic acid conjugate (Billing, Cole and Lathe, Schmid). Failure of this conjugation, transient or permanent, has been suggested to account for (1) physiological jaundice (Billing and Lathe); (2) jaundice found in a strain of rats and due to a recessive gene (Carbone and Grodsky), and (3) familial nonhemolytic jaundice (Schmid). We have tested this hypothesis by studying the capability of two patients with the latter disease to make glucuronic acid conjugates of several substances by administering the substances and measuring the specific glucuronides in the urine. One patient was tested with radioactive hydrocortisone, trichloroethanol, and sodium salicylate, and the second patient with salicylate alone. In each case the patient exhibited a markedly diminished ability to conjugate the test substance with glucuronic acid, the values obtained being well below the lowest given by the controls.

Since the disease is due to a recessive gene all parents must be heterozygotes, and some sibs may be. In all, 19 relatives from 4 families containing at least 1 affected child were tested for their ability to conjugate trichloroethanol or salicylate. Of these, eight were parents. Two parents were given trichloroethanol and both excreted the glucuronide in amounts below the lower limit of the controls but above those excreted by the patients. Seven of the parents excreted salicylate glucuronides in amounts less than normals but greater than the patients. In addition, there were four sibs and one grandmother who by this test could be marked as heterozygotes with a high degree of probability.

Both ester and ethereal glucuronides of salicylic acid are excreted in the urine (Kapp and Coburn, Schacter). The ester form is hydrolysed by alkali and can be measured as free salicylate after hydrolysis. Approximately equal amounts of the two glucuronides are excreted by the normal control. On the other hand most, and frequently all, of the salicylate glucuronide excreted by patients and parents was of the ester type. It has been agreed that bilirubin glucuronide is of the ester variety (Billing, Cole and Lathe, Schmid, Schacter). Our data, however, suggest that the defect in familial nonhemolytic jaundice is a general impairment in glucuronic acid conjugation involving both types of glucuronide. Neither hydrocortisone nor trichloroethanol possess a carboxyl group with which to form an ester and in the patients and some of the parents, the formation of the ethereal glucuronide of salicylate was even more depressed than was that of the ester.

50. Further Studies in Glucuronide Conjugation in the Newborn

AUDREY K. BROWN*; WOLF W. ZUELZER, and ALFRED JAY BOLLET,* with the Assistance of HERBERT H. BURNETT, Wayne

The principal pathway for glucuronide conjugation, including the formation of bilirubin glucuronide (direct-reacting bilirubin), involves a series of enzymatic steps effecting the transfer of an active glucuronic acid moiety from uridine diphosphate glucuronic acid (UDPGA). We recently demonstrated that the liver of the fetus and newborn were deficient in the activity of two enzymatic steps in this system (UDPG dehydrogenase and glucuronyl transferase). These deficiencies appear to be responsible for the accumulation of unconjugated (indirect-reacting) bilirubin in the newborn.

Sites of extrahepatic glucuronide synthesis were studied in an effort to survey the total potential for this system in the newborn. Heart, lung, and spleen failed to shown glucuronyl transferase activity, but kidney glucuronyl transferase was found to develop in parallel with the liver enzyme.

UDPG dehydrogenase, which converts UDPG to UDPGA was found in homogenates of human placenta and glucuronyl transferase activity was demonstrated in fraction from placenta prepared by ultracentrifugation at 80,000 g.

Alternates to the accepted pathway of glucuronide synthesis were also investigated since augmentation of these might enhance bilirubin excretion by the newborn. Danoff and Holt have reported that glucuronic acid administration lowers serum bilirubin levels in the newborn. Preliminary studies in our laboratory with glucuronolactone

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have suggested that some decrease may result, but these studies were pursued in vitro rather than in vivo.

The effect of glucuronic acid and related substances on in vitro glucuronide conjugation was surveyed. Liver homogenates from newborn and adult guinea pigs were incubated with sodium glucuronate, glucuronolactone, glucuronic acid, glucose, or glucose-1-PO_4. No conjugation could be demonstrated with any of these substances unless ATP was added, in which case glucuronic acid, sodium glucuronate, and glucuronolactone produced a definite conjugation of orthoaminophenol. Other β-glucuronidase inhibitors such as saccharic acid and mucic acid could substitute for sodium glucuronate when ATP was also added. No conjugation could be demonstrated with glucose even after addition of ATP. Glucose-1-PO_4 plus UTP could be substituted for ATP.

These observations require consideration of the role of β-glucuronidase which is known to be increased in the newborn. β-glucuronidase added to liver homogenate plus UDPGA decreased the amount of demonstrable conjugation of orthoaminophenol; sodium glucuronate reversed this influence. This data prompts a study of the possible modes of interaction between the UDPGA transferase system, ATP, glucuronic acid, and β-glucuronidase.

51. The Demonstration of Antibodies to Thyroglobulin (Autoimmunization Phenomenon) in Patients with Thyroid Disease

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Precipitin antibodies to human thyroglobulin recently have been demonstrated in a majority of patients with Hashimoto's thyroiditis. Antibodies to thyroglobulin also have been demonstrated in animals injected with thyroid extracts. A study was therefore undertaken to determine the frequency of antibody production occurring in various types of thyroid disease. Antibodies were determined by a RBC hemagglutination technique using pure human thyroglobulin as the antigen. The results are tabulated below.

	Total	Positive	Negative
Thyrotoxicosis	47	16	25
Nontoxic nodular goiter	11	5	6
With hypothyroidism	2	0	2
Without hypothyroidism	9	5	4
Spontaneous myxedema	3	3	0
Hashimoto's thyroiditis	2	0	2
Subacute thyroiditis	1	0	1
Acute thyroiditis	1	0	1 (4 mo.)
Thyroid carcinoma	2	0	2
Athyrotic cretinism	11	1	10
Chronic thyroiditis	1	1	0
Adolescent goiter	1	0	1
Controls (patient with no apparent thyroid disease)	123	2	121
Normals treated with I^{131}	1	0	1

This study suggests that release of thyroglobulin locally or systemically secondary to thyroid injury or hyperplasia of any etiology is all that is required for autosensitization to occur.

The presence of antibodies, for example in thyrotoxicosis, was not related to the administration or type of therapy. In addition, it is suggested that auto destruction of the thyroid may be a contributing factor in spontaneous myxedema. This hypothesis is supported by the demonstration of a lymphoid infiltration in the thyroid of all patients thyroidectomized having circulating antibodies.

Tangential studies were also performed. An elevated globulin level was usually found in patients not on thyroid therapy and with demonstrable antibodies. Therapy reversed this finding. The thymol turbidity, however, was not usually elevated. Cortisone in vivo or in vitro had no effect on the demonstration or titer of antibodies.

Further studies in humans and animals are in progress.

* By Invitation

52. Giardiasis Causing the Celiac Syndrome; Clinical Observations and Studies of I^{131} Fat Absorption

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Introduced by RUSTIN MCINTOSH.

Four patients with a celiac-like syndrome have been studied, in whom Giardia lamblia infection was discovered and for whom a cure was effected by treatment with atabrine.

The first two cases (a 3-year-old white girl and a 19-month-old white boy) were studied at Vanderbilt Hospital in 1956. In each there was a history of typical foul greasy stools and failure to thrive despite large appetite; physical findings of muscle wasting and protuberant abdomen; and laboratory studies which demonstrated steatorrhea and malabsorption (by vitamin A tolerance test) and which failed to establish any other cause in steatorrhea. One patient showed a megaloblastic anemia. In both patients microscopic examination of the duodenal aspirate revealed numerous active forms of Giardia lamblia, though in one case cysts were difficult to demonstrate in the stool. Both children were treated with atabrine for five days, following which steatorrhea subsided, the abdomens became less protuberant, and weight gain ensued.

Cases 3 and 4 (a 3-year-old white girl and a 22-month-old Negro boy) were studied at Babies Hospital in 1956-7. Again, both had historical and

Percent Ingested I^{131}-Labeled Fat in the Serum

Date Hr. After Ingestion	Case 3		Case 4			
	3/7/57	5/1/57	1/9/58	4/30/57	9/24/57	Normal
2	0.8	1.7	1.8	1.6	3.7	3.8
4	1.7	2.4	3.3	3.6	3.6	5.5
6	1.6	1.8	3.2	2.1	2.1	5.8
24	1.8	0.6	1.6	1.0	1.0	1.8

physical evidence of celiac syndrome and laboratory studies which demonstrated steatorrhea and failed to support other causes, such as fibrocystic disease, celiac disease, or colitis. Microscopic examination of the duodenal aspirate revealed Giardia lamblia, and again not all stools examined contained cysts. In addition to other laboratory studies, an I^{131} labeled fat absorption test (by the method of Malm, Reemtsma and Barker) was performed before and after treatment of the giardiasis with atabrine for five days.

In Case 3 there was marked reduction of absorption before treatment (see Table), moderate improvement one month after treatment, and further improvement nine months after treatment. In Case 4 only moderate improvement was demonstrated five months after treatment.

Both of these patients, like the earlier ones, experienced complete clinical remission of celiac-like symptoms.

Two other cases (a 5-year-old Puerto Rican boy and a 7-year-old Puerto Rican girl) of low-grade recurrent abdominal pain, with no clinical evidence of malabsorption but with Giardia infection found during a routine investigation, were studied by I^{131}-labeled fat absorption test before and after treatment with atabrine. In one, though the serum levels of I^{131} fell, the urinary excretion of the isotope improved; in the other, the serum levels were low both before and after treatment, and urinary excretion studies were not made. In this last case abdominal pain persisted even though Giardia could no longer be demonstrated in the stool.

It may be concluded that a rare cause of celiac syndrome in childhood is giardiasis (as has been occasionally described in the literature), that numerous active forms of Giardia lamblia can be demonstrated in duodenal aspirate even when cysts are not constantly present in the stool, and that the syndrome is clinically reversible with atabrine treatment.

53. A Familial Metabolic Disorder with An Unusual Polysaccharide Complex

JOHN M. CRAIG and L. LAHUT UZMAN,* Harvard

In this report we describe a new form of metabolic defect with storage of an unusual complex of polysaccharide.

* By Invitation

Of the three cases reported, two were siblings. The first, a girl, was born prematurely, and developed anemia at 6 months and a marked hepatosplenomegaly at 13 months of age. Leukopenia and thrombocytopenia were also present. Blood chemical studies revealed normal cholesterol values, positive liver flocculation tests, and a flat blood glucose curve after epinephrine. Splenectomy relieved the pancytopenia. Five weeks after operation she was readmitted because of persistent anemia. A pericardial effusion developed, which caused death by cardiac tamponade at 25 months of age.

The male sibling of this child developed fever and a sterile pericardial effusion after removal of a lipoma at three and a half months of age. At 11 months of age he was poorly developed and had a greatly enlarged liver and palpable spleen. He died at 23 months of age.

The third case was a white male seen at age nine months because of a hernia, anemia, and a greatly enlarged liver and spleen. The blood glucose response to epinephrine was negligible. A liver biopsy was performed and the hernia repaired. The child was not seen again, but he died at 21 months of age.

In the first case, the surgically removed spleen, the lymph nodes, and, at autopsy, the interstitial areas of the heart, kidneys, bladder, alveolar spaces in the lungs, and perivascular spaces in the brain contained foamy macrophages. The parenchymal cells in the cirrhotic livers in the first and third cases were vacuolated and contained large crystalline masses while in the fibrous trabeculae and in the sinusoids were numerous macrophages with vacuolate translucent slightly basophilic cytoplasm.

Histochemical procedures of the liver specimens gave positive reactions with Best's carmine, MacManus PAS, and the Hale stain in the macrophages and crystalline masses. A dark blue reaction was obtained with iodine. The PAS reaction was not diminished by malt diatase, ptyalin, takadiastase or by 1 normal hydrochloric acid. Acetylation blocked the reaction after 24 hours of treatment; it was restored with 0.1 potassium hydroxide. The crystalline deposits of the liver were negative for lipid, though the remainder of the liver cell cytoplasm was positive.

Chemical examination of the liver in Case 1 showed no significant increase in the lipid content of the liver. After homogenization in dilute 1:1 formaldehyde, the homogenate was filtered, the filtrate dialyzed, concentrated, and acidified to pH 6.5. Alcohol was added in equal parts and a precipitate obtained (Fraction I). A second portion was precipitated with addition of two more volumes of 95% alcohol (Fraction II). The remaining supernatant was treated with 5 volumes of cold acetone and the precipitate formed was Fraction

III, a greenish powder, soluble in water or alcohol, but not in ether or chloroform. Fractions I and II were hydrolyzed and chromatographed. A high proportion of Fractions I and II were found to contain glucose, galactose, glucosamine, and hexuronic acid. Peptides were closely bound to these fractions. Methylation followed by chromatographic procedures indicated that the galactose elements probably were terminal units of the side chains. Fraction III was considered to be of the ganglioside class because of its solubility in water as well as alcohol, and its content of neuraminic acid.

54. Studies of Familial Oxalosis

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Introduced by Robert A. Aldrich.

A seven-year-old boy with recurrent pyuria and calcification of the renal pyramids was found to have markedly elevated urinary oxalate excretion (average, 175 mg/25 hr.). Except for polyuria, his general health and growth have been normal. No renal calculi were demonstrated initially. Other members of the family also have elevated urinary oxalate values (over 50 mg. per day) but no evidence of clinical disturbance. Hyperoxaluria was found in three generations, and the pattern of inheritance suggests dominance.

The following studies were carried out in an attempt to determine the source and metabolic pathway of the abnormal urinary oxalate.

1. The serum oxalate was found to be 9μg. per milliliter, whereas the four controls were less than 5μg. per milliliter. Slit-lamp examination of the cornea and aspiration of the bone marrow revealed no crystals.

2. Dietary oxalate was reduced to less than 50 mg. per day for a period of 14 days with no change in his total daily urinary oxalate.

3. The effect of dietary carbohydrate was determined by a high carbohydrate diet. No change was observed.

4. There was no correlation between the vitamin C content of his diet and the urinary output. Intravenous loading with 100 mg. of ascorbic acid did not increase the oxalate output.

5. The most likely source of oxalate was from protein metabolic pathways as suggested by the following findings:

a. The serum uric acid was elevated without azotemia.

b. His output of oxalate increased about 50 mg. per day when placed on a high protein diet.

c. Even when the total urinary nitrogen was reduced by 50% the urinary oxalate was between 3% to 5% of the total nitrogen.

* By Invitation

55. Defect of the Iodinating System in Congenital Goitrous Cretinism: Report of One Case with Biochemical Studies

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Johns Hopkins

A 15-year-old male with severe goitrous cretinism had a high I^{131} uptake by his thyroid gland, which was sustained over 72 hours but was completely dumped with oral thiocyanate. Although the patient failed to dehalogenate exogenous I^{131}-labeled diiodotyrosine, there were no significant amounts of iodinated tyrosines or thyronines in his plasma or urine, when studied by paper chromatography after I^{131} administration.

Thyroid tissue, obtained by partial thyroidectomy, was homogenized in Krebs-Ringer's bicarbonate buffer, was fractionated by differential centrifugation, and the 900 g and the 30,000 g fractions were incubated with I^{131} and iodide, chromatographed on paper (in butanol-ethanol-ammonia) and radioautographed. Radioactivity was then determined in a well-counter.

Three radioactive spots were formed on the paper chromatogram: (1) that at the origin, O; (2) that which migrated with the iodide, I, and (3) an unknown, U, which had an R_f value similar to thyroxine. Most of O component disappeared on acidification, and it almost completely disappeared upon digestion with pangestin. No iodinated tyrosine was obtained; the disappearing radioactivity moved quantitatively to the I spot. Additional studies showed that iodic acid and iodates remain at the origin of the paper chromatogram, and that both were acid-labile, liberating elemental iodine, which had the same R_f value as iodide. The U spot, on the other hand, completely disappeared with acidification, digestion with pangestin, and when treated with thiocyanate at the termination of the incubation; its radioactivity being quantitatively added to that of the I spot. Its nature still remains unknown.

When normal thyroid tissue was treated in a similar way, the same distribution of three radioactive spots appeared. The O component was approximately 75% acid stable, and liberated iodinated tyrosine upon pangestin digestion. The U spot, however, behaved the same as with the goitrous cretin.

When glucose and xanthine oxidases were incubated alone with their respective substrates, iodide and I^{131}, the same three spots appeared on the paper chromatogram; O and U had characteristics similar to those produced by the patient's thyroid tissue. The addition of glucose or xanthine oxidase to the incubation mixture of the patient's thyroid tissue caused no quantitative or qualitative change in the radioactive components obtained; whereas,

there was about 50% increase in the O component produced by the normal thyroid tissue when incubated with glucose or xanthine oxidase.

The defect in the patient's thyroid gland, therefore, seems to be an inability to convert iodide into organic form, and hence no iodination of tyrosine occurs.

56. Study of the Effects of a Therapeutic Attempt at Complete Parenteral Alimentation in Infants

LeRoy W. Matthews*; Wayne H. Borges*; Robert P. Bolande,* and Samuel Spector, Western Reserve

Five consecutive infants with severe problems in oral alimentation were given glucose, fructose, amigen, fat, minerals and vitamins intravenously in an attempt to provide adequate nutrition. All five showed adverse hematologic effects.

Three of the infants, two with marked malnutrition secondary to chronic diarrhea and one with a congenital defect of the small intestine, were intensively studied during the period of therapy, which lasted from 5 to 24 days. Determination of serum lipids, fat clearances, liver function, nitrogen, sodium, potassium and chloride balances, and hematologic studies were performed. The two infants with chronic diarrhea who had previously failed to respond to our usual therapy of withholding oral intake for a short period, administration of parenteral fluids and a gradual return to oral feedings, subsequently showed a return to normal gastrointestinal function. All three infants during therapy gained weight, supported in two by a positive nitrogen balance, the third remaining in nitrogen equilibrium despite fever. All three developed hepatosplenomegaly, marked hemolytic anemia, thrombocytopenia and leucocytosis. The two tested showed increased osmotic fragility of their red blood cells. These changes were evident as early as the fifth day of therapy.

Two of the infants with severe chronic disorders in whom intensive studies were not performed also showed hematologic changes. These two infants and the child with the congenital defect of the small intestine expired and autopsies were performed. In all three there was evidence of hemosiderosis in the liver and spleen. Lipid disposition was most pronounced in the livers where small intrasinusoidal "lipoid granulomas" were seen in close association with hemosiderin deposits.

57. Nitrogen Utilization in Caloric and Protein Depleted Infants

Mitchell I. Rubin; P. K. Mukherji,* and Philip L. Calcagno, Buffalo

The effect of caloric intake on protein utilization and tissue synthesis has been a subject of wide debate over many years. Rubner demonstrated improved utilization of protein when the caloric intake was maintained. However, in clinical practice, when parenteral fluid therapy is employed as the sole source of calories, it is often difficult to maintain optimal caloric intake. It seemed, therefore, important to determine the nitrogen retention in infants while on an inadequate caloric intake. Cox, Ellingson and Mueller showed that under conditions of protein depletion in rats there was an increased utilization of ingested nitrogen for protein synthesis even during low caloric feeding.

Retention of parenterally administered nitrogen was determined in infants who were protein starved and on low caloric diets for four to five days prior to testing. Because of their large protein needs for growth, it was decided to use infants rather than older children in this study. A group of five infants was fed a protein-free diet at a caloric intake of 38 Cal. per kilogram body weight per day for four to five days (Period I). This period was followed by a four to five day period when parenteral nitrogen administration at a rate of 100 mg. per kilogram body weight per day was given in addition (Period II). A final depletion period (III), similar to Period I, was obtained to determine the extent of nitrogen losses in the after-period.

During the three periods studied, the infants were fed approximately at isocaloric levels (38-41 Cal. per kilogram body weight per day). The results expressed on the basis of body weight for the entire group of infants show the following average nitrogen balance data:

Period I—133.6 mg/kg/day (Nitrogen-free period)
Period II—23.1 mg/kg/day (Parenteral nitrogen administration)
Period III—55.3 mg/kg/day (Final nitrogen-free period)

Three of the five infants during Period II were in positive nitrogen balance.

Data on the plasma concentrations and renal excretions of urea and amino nitrogen obtained during these balance periods will be presented. Interpretation of these data suggest utilization of this retained nitrogen during deficient caloric intakes.

Conclusions were as follows:

1. The administration of amino acid nitrogen parenterally to infants who are underfed (40 Cal. per kilogram) and protein-starved can restore the negative nitrogen balance to a lesser nitrogen or even a positive nitrogen balance.
2. The retained nitrogen is not degraded to urea or excreted as amino acid nitrogen in the urine in the after-period. It is postulated that the retained amino acid nitrogen is utilized for synthesis.
3. The use of parenteral amino acid feeding in prolonged protein starvation in infants who are

* By Invitation.

unable to ingest protein seems desirable, even when the total caloric intake as carbohydrates is at basal levels.

58. Growth and Body Composition of Piglets on Various Feedings

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Direct study of the relationship between diet and body composition of the human infant is practically impossible. Chemical analysis is limited to postmortem specimens, where dietary intake is inadequately recorded and body composition is frequently distorted by disease.

Since failure of the albino rat to thrive on human milk has been established, it seemed worthwhile to study the growth and body composition of piglets raised from birth to eight weeks of age (weaning) on diets resembling those frequently fed to human infants. Such studies might allow conclusions to be drawn on the value of the pig as an experimental animal in nutritional research related to infants.

Body composition was established by determining the specific gravity of the eviscerated carcass and by chemical analysis of the carcass for water, fat, ash, and nitrogen.

The basal diet (Diet I) consisted of powdered infant formula containing 13.8% protein, 26.8% fat, 53.4% lactose, and 4.0% ash, to which were added traces of iron, copper, and manganese. Diet II was prepared from the basal diet by the addition of extra quantities of the water- and fat-soluble vitamins, and liver concentrate; Diet III by adding casein to increase the weight by 10%, and Diet IV by addition of the vitamin mixture and the casein.

These diets provided approximately 45% of the Calories in the form of vegetable oils and 11% or 17% of the Calories as protein. Groups of three pigs each were placed on each diet.

Results indicate that the suckling pigs grow well on diets similar to those fed human infants. In contrast to the rat, they tolerate the high lactose content of the diet without developing diarrhea.

* By Invitation.

Since the additional vitamin supplement did not alter body composition, the groups on Diets I and II, and on Diets III and IV, were combined. Average body composition for the combined groups is as follows.

	Water		Fat			
	Chem.*	Sp. Gr.*	Chem.†	Sp. Gr.*	Protein ‡	Ash
Groups I and II (11% of Cals. from protein)	51.4	46.3	31.7	37.8	18.1	3.0
Groups III and IV (17% of Cals. from protein)	54.1	47.6	28.7	36.3	18.4	2.9

* Difference not significant.
† Difference significant at 5% level.
‡ Calculated on fat-free basis, difference not significant.

These findings on body composition compare with those reported by Widdowson and McCance, and by Wallace and Taylor, for young rats fed diets with varying protein content.

59. The Febrile Seizure Threshold in Relation to Maturation of Brain Water, Electrolyte, and Acid-Base Balance of Newborn Animals

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Seizures with hyperthermia were induced in mice, rats, and guinea pigs by means of a microwave diathermy generator. Responses were more uniform than those obtained with radiant heat. Groups of mice aged three to four weeks were subjected to five different intensities of heating current between 30% and 100% of the maximal power output. The initial basal body temperature and temperature at onset of the generalized clonic seizure were recorded with a thermistor hypodermic probe and telethermometer. The temperature at which the convulsion occurred was constant at 110 F in all groups while the rate of rise of body temperature varied from 2 to 8 F per minute. In mice subjected to weaker stimuli, the maximal body temperature attained was 105-108 F and seizures were not induced. Furthermore, the rate of rise in temperature could be altered by certain drugs (e. g., chlorpromazine) without modification of the convulsive temperature. Thus, the height of the body temperature was the determining factor in the induction of seizures with hyperthermia. A febrile seizure threshold related to the height of the fever was observed also in a clinical study of 114 patients.

In the laboratory procedure described, the convulsive temperature provides an accurate measurement of the febrile seizure threshold. In subsequent experiments, the threshold convulsive temperature was determined in relation to age and biochemical maturation of the brain. In newborn rats during the first week of life, the threshold was approxi-

mately 107 F and between 12 and 28 days, 109-110 F. In older rats, the seizures were milder and the threshold was markedly raised; the convulsive temperature of 114-116 F was close to that at which the animals died. The low convulsive temperature of the newborn rat was unrelated to its low basal body temperature. In the newborn guinea pig, an animal more mature at birth, the febrile seizure threshold was equal to that of the 20-day-old rat.

In order to elucidate the mechanism of febrile seizures, the increased susceptibility of the newborn compared with older rats was studied in relation to the maturation of water and electrolyte distributions and acid-base chemistry of the brain. In rats aged four to seven days, the total brain sodium and chloride concentrations were 30% higher and the brain potassium, 15% lower than in one month old and adult rats. The plasma potassium was high and the sodium unchanged in newborn compared with older rats. The brain water content (88%) was high and the ratio of cellular to extracellular water (1:4) was relatively low in newborn animals. There was evidence of a direct relation between the total and the ratio of cellular to extracellular potassium in the brain and the febrile seizure threshold. A relatively low total and cellular potassium concentration was associated with a high total CO_2 content of the brain of the newborn rat. The brain tissue pH of 6.86 in the one month old rat was not significantly different in the newborn. Developmental variations in the total brain sodium and chloride concentrations were related inversely to the febrile seizure threshold.

Acetazolamide, which increases the cellular and the ratio of cellular to extracellular potassium in the brain, caused a rise in the threshold convulsive temperature. Diphenylhydantoin, which fails to modify cellular potassium concentration, had no effect on the threshold temperature and even exacerbated the clonic febrile seizure. Hypoelectrolytemia and the decrease in total and in cellular brain potassium which follows the intraperitoneal injection of isosmolar glucose was associated with a fall in the febrile seizure threshold. In rats treated with isotonic saline, no change in the threshold was observed, while in animals deprived of water and dehydrated, an elevation of the threshold convulsive temperature was obtained. These laboratory data and a clinical observation of hyponatremia in children with febrile seizures suggest the inadvisability of rapid hydration and use of hypotonic parenteral solutions in therapy.

60. Studies on the Mechanism of Production of Nephrosis by Aminonucleoside

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Introduced by W. M. Wallace.

The nephrotic syndrome can be regularly induced in rats by the aminonucleoside of puromycin, 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine, which will be designated the dimethyl adenosine derivative. This nucleoside failed to produce the disease in dogs, guinea pigs, rabbits, and mice. The following studies of the urinary metabolites appearing after injection of the nucleoside were undertaken to clarify the mechanism of its action.

It had been shown previously that neither the free base, 6-dimethyl-amino purine, nor the sugar, 3-amino-3-deoxy-D-ribose, was nephrotoxic for rats. When the free base was injected into rats it could not be recovered in the urine by ion exchange chromatography. When the dimethyl adenosine derivative was injected into rats, rabbits and guinea pigs, mixtures of nucleosides were isolated from the urine but no free base was found. These mixtures of nucleosides were separated by paper chromatography. The following results were obtained: 1. All animals excreted some of the dimethyl adenosine derivative unchanged. 2. All animals were able to convert the dimethyl adenosine derivative to the monomethyl adenosine derivative [6-methylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine]. 3. All animals converted the dimethyl adenosine derivative, and the rat and rabbit converted the monomethyl adenosine derivative to compounds with substitution probably on the sugar. Preliminary experiments suggested that this substitution was not an acetylation of the nitrogen. 4. The rat and rabbit both converted the dimethyl adenosine derivative to the adenosine derivative [6-amino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine]. 5. The rabbit converted the dimethyl adenosine derivative to the isonine derivative [6-hydroxy-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine].

The presence of the unchanged dimethyl adenosine derivative in the urine of all animals excluded the possibility that the rabbit and guinea pig were able to degrade the compound and therefore did not develop the disease. This also suggested that the unchanged nucleoside did not cause the disease.

Unlike the guinea pig, the rat and rabbit could degrade the compound as far as the adenosine derivative. However the rabbit could convert this to the isonine derivative. This compound was found to be nontoxic in rats. It seems possible that the active compound is the adenosine derivative.

* By Invitation.

61. Alterations in Bone Composition Following Nephrectomy in the Rat

WILLIAM H. BERGSTROM and EUGENE R. JACOBS,* State University of New York College of Medicine at Syracuse

The importance of the skeleton as an electrolyte reservoir has been shown by numerous investigators within the past decade. The role of bone as a buffer in acidosis has also been indicated. The critical factors essential to release of bone base remain obscure, since most experiments have involved the simultaneous production of acidosis and renal or dialytic base loss. The present experiments demonstrate the effects of bilateral nephrectomy on bone composition. Rats thus made acidotic show striking reductions in bone sodium, potassium, calcium, and carbonate, although no significant loss of total body cations occurs. After simultaneous parathyroidectomy, bone calcium loss is diminished but the observed decrease in univalent cations is greater than with nephrectomy alone. The data suggest that pH change alone suffices for release of bone sodium and potassium, whereas parathyroid activity is essential to maximal calcium mobilization. Implications regarding the association of sodium and potassium with carbonate in bone will be discussed.

The dissolution of bone in acute renal insufficiency involves a large phosphate shift, since the observed bone carbonate decrement by no means covers the changes in calcium, sodium, and potassium. It is apparent that hyperphosphatemia in anuria may be even more dependent on osteolysis than on catabolism of protoplasm.

62. Total Body Water and Bromide Space Determinations in Premature Infants

NED W. SMULL,* Kansas
Introduced by HERBERT C. MILLER.

Previous investigators have called attention to the fact that some premature infants appear edematous at birth. It has been suggested from clinical observations that the edema is accompanied by respiratory distress and is possibly prejudicial to survival. A method has been previously devised in this clinic for early recognition of respiratory insufficiency in newborn infants by observing the trend of the respiratory rate following birth. The purpose of the present study was to determine total body water and bromide space in premature infants and determine if there was a correlation between their volumes and the presence of respiratory insufficiency.

Total body water and bromide space were determined on 21 infants. The total body water was estimated by dilution of deuterium oxide (method of Schloerb, Friis-Hansen, Edelman, Sheldon, and Moore). Bromide space was determined by the bromide dilution method of Hellerstein and Darrow.

Infants were classified into three groups according to the trends of their respiratory rates. Infants in Groups I and II did not have severe respiratory insufficiency; infants in group III did have severe respiratory insufficiency. Studies to date show that mean total body water was 78% in 5 infants in group I, 80.6% in 10 infants in group II, and 88.2% in 6 infants in group III. Mean bromide space was 45%-55% in 4 infants in Group III, in comparison to 40% in 12 infants in Groups I and II. While total body water showed some inverse correlation with the birth weights of the infants, the more striking positive correlation was with the degree of respiratory insufficiency. These data suggest a relationship between total body water and severe respiratory insufficiency in premature infants.

63. Metabolic Alkalosis: A Specific Effect Of Adrenocortical Hormones

A. P. GROLLMAN * and J. L. GAMBLE JR., Johns Hopkins

Studies were undertaken to evaluate the relative importance of adrenocortical hormones and potassium deficiency in the etiology of metabolic alkalosis. Our analyses of reported clinical data indicate that alkalosis (CO_2 greater than 33 mEq. per liter) induced by adrenocortical activity was usually accompanied by only moderate potassium deficiencies (less than 6 mEq. per kilogram) whereas larger deficiencies (greater than 8 mEq. per kilogram) have been reported in subjects in which the CO_2 elevations were minimal (CO_2 less than 33 mEq. per liter).

With appropriate balance experiments and muscle analyses, it was shown that: 1. Dogs placed on a potassium-deficient diet alone for five months did not develope alkalosis. 2. The administration of DOCA, ACTH, or cortisone to animals on a potassium-deficient diet evoked metabolic alkalosis which was sustained only as long as the hormones were administered. A prompt return to normal occurred despite continued potassium restriction. 3. Alkalosis induced by chloride withdrawal or by cortisone administration during complete sodium restriction was not associated with evidence of potassium loss.

The findings have led us to conclude first, that there does not appear to be an obligate relationship between extracellular alkalosis and intracellular potassium deficiency; and second, that the adrenocortical hormones can induce alkalosis quite independently of effects upon potassium metabolism.

* By Invitation.

64. Meningitis in Newborns Caused by An Unclassified Gram-Negative Bacterium

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Introduced by HEINZ EICHENWALD.

Outbreaks of meningitis caused by a previously undescribed Gram-negative organism have occurred in hospital nurseries in Greenville, South Carolina and Portsmouth, Virginia during the past two years. This organism which has not yet been successfully classified, has also been isolated in Florida, Massachusetts, Pennsylvania, North Carolina and Georgia. Of the 19 cases in the two outbreaks, 17 were in premature infants. Sixteen became ill between the 5th and 9th day of life. The clinical course was characteristic for bacterial meningitis in this age group, with a few infants succumbing early of sepsis and several progressing to hydrocephalus. Sixteen of the patients died. Age, sex, weight, condition at birth, treatment, and medication were not found to be predisposing factors. A Gram-negative bacillus was recovered from the spinal fluid on 17 occasions. Seven cultures which were available for study contained a long pleomorphic nonmotile organism, not fastidious in growth requirements which is oxidase positive, a weak indole producer which will ferment certain carbohydrates after a week. It is highly resistant to antimicrobial agents. The bacillus has been recovered from the throats of healthy infants in the nurseries, but, thus far, never from adults or the physical environment. Although the definitive epidemiology has not been worked out, there is the strong suggestion that this represents a new form of hospital infection.

65. The Use of Latex Particles in the Serological Study of Bacterial Antigen-Antibody Systems

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Singer and Plotz recently introduced a latex fixation or agglutination test for the serological diagnosis of rheumatoid arthritis and reported that the technique could also be used with Type VII antipneumococcal rabbit serum and Type VII pneumococcal polysaccharide. At that time we were working with a group of anaerobic diphtheroids, and we were experiencing difficulty using standard bacterial agglutination tests because of apparently weak reactions and antigens which often agglutinated spontaneously. We therefore attempted to apply the latex fixation technique to this problem. The results were so satisfactory that we are reporting the technique we evolved for bacterial antigen-antibody systems.

An antigen-latex mixture is prepared by mixing a suspension of bacteria, latex, and glycine saline buffer (pH 8.2). The latex particles are the uniform 0.81μ polystyrene particles used in the test for rheumatoid arthritis, but the stock solution is diluted 1:4 with buffer. To yield 5 ml. of this mixture, 0.5 ml. of the antigen, 0.2 ml. of the latex suspension, and 4.3 ml. of buffer are mixed and incubated for one hour in a 56 C water bath. Sera are diluted two fold starting with a 1:10 dilution in glycine saline buffer to yield 0.5 ml. in each tube. Clear test tubes measuring 10×75 mm. are used. To each tube of diluted serum, 0.5 ml. of the prepared antigen-latex mixture is added, the tubes are shaken, incubated for one hour in the 56 C water bath, centrifuged at 2300 rpm for three minutes, and read. Appropriate serum and antigen controls are included. Positive reactions are indicated by a water clear supernatant, negative reactions by a turbid supernatant similar to that in the control tubes. Adding the latex first to the serum dilution rather than to the antigen led to uniformly negative results.

With this technique it was possible to show that a rabbit serum prepared against one of the anaerobic diphtheroids gave positive reactions with all anaerobic diphtheroid strains recovered from patients and with a known anaerobic diphtheroid. A strain of actinomycetes which in a number of ways resembles anaerobic diphtheroids failed to react with this serum. There was no correlation between the antibody titers obtained in agglutination tests and latex-fixation tests.

We have also shown that this technique may be used with young cultures of H. influenza type B and a specific rabbit antiserum.

Because of its simplicity and ease of reading the latex-agglutination test shows promise of becoming a useful tool in the serological study of certain infections.

66. Host-Graft Relationships Using S^{35} Methionine

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MILTON T. EDGERTON JR.*;
HARVEY KEMP JR.,* and
WILLIAM SEIP,* Johns Hopkins

Two experiments were carried out to obtain information concerning possible movement of tissue protein between skin homotransplant and the host. In the first of these, skin transplants from donor mice labeled 24 hours before by administration of S^{35} methionine were transferred to homologous mice. At intervals up to 15 days the host mice were killed and the organ proteins' radioactivity was measured. Distribution of isotope was that usually found after feeding amino acids and gave no indication of dissemination of graft proteins to the host.

* By Invitation.

In the second experiment, a skin graft from a homologous mouse was transplanted to a host earlier labeled with S^{35} methionine and after 24 hours the host mouse was sacrificed. The proteins of the transplanted graft had achieved an isotope concentration equal to that of the skin of the host. The same was found true for an isologous skin transplant in 24 hours. Further studies are under way.

67. The Nature of "Immunological Paralysis" Induced by Pneumococcus Polysaccharides

DAVID GITLIN; FERNANDO MONCKEBERG,* and JOHN M. CRAIG, Harvard

In 1949, Felton discovered that, while small doses of pneumococcus capsular polysaccharide induced type-specific immunity in mice against pneumococci, large doses failed to do so. Since the polysaccharides persist in tissues for many months, it has been widely accepted that this immunological failure is due to the rapid removal of synthesized antibody from the circulation by the persisting polysaccharide. Because this interpretation seemed untenable, the problem was reinvestigated. First, the effect of antibodies on the persistence of pneumococcus polysaccharide was determined. Normal mice were injected with various amounts of rabbit antibody and then given a specific load of polysaccharide. It was found that as the amount of antibody present in the mouse increased, the amount of polysaccharide in the circulation decreased; this finding provided a sensitive test for specific antibody production. Then, the effect of the size of polysaccharide load on antibody production was studied: groups of mice were given either large doses of polysaccharide, 0.5 mg., or immunizing doses, 0.005 mg. The serum disappearance of polysaccharide was followed and histological examination of the lymph nodes for changes characteristic of antibody production was performed. Significant signs of antibody production following the large load of polysaccharide could not be found. A second injection of polysaccharide, 0.025 mg., was given these mice a month after the first injection and again the serum disappearance of polysaccharide was followed and histological studies made. It was observed that even following this booster injection, antibody production in those mice given the large load was still absent and only poor even in those mice given immunizing doses. Thus, (1) pneumococcus polysaccharide at best is a poor antigen in the purified state, and (2) specific antibody synthesis is actually inhibited by large amounts of polysaccharide.

* By Invitation.

68. Scrofula Caused by Chromogenic Acid-Fast Mycobacteria

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Introduced by EDWARD L. PRATT.

Previous reports have indicated that young children may exhibit scrofulous lymph nodes of the neck caused by atypical acid fast organisms. The present report suggests that this entity may have a pathogenesis and clinical course distinguishable from scrofula caused by the human tubercle organism. Atypical organisms have striking predilection for nodes at the angle of the mandible. Inflammation seems to be more acute, and suppuration takes place earlier. There is definite preponderance in white children between one and four years of age; a possible mode of entry may be through gum or tonsil. Although the tuberculin test is usually positive, history of tuberculosis contact is lacking. Chest films are usually not significant.

A hypothesis that these organisms represent mutations from Mycobacterium tuberculosis is not substantiated, since the organisms isolated from biopsied nodes are derived from six previously untreated children.

It is further evident that these organisms differ from each other as they do from M. tuberculosis. Isolated organisms belong to each of the three major groups suggested by Runyon. Sensitivity to antituberculosis drugs, neutral red reaction, peroxidase activity and animal pathogenicity studies vary with each strain, although histological reaction is identical among all strains and identical with that to M. tuberculosis.

69. Isonicotinic Acid Hydrazide Levels in Children

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Isonicotinic acid hydrazide (INH), the most important antituberculous antimicrobial drug at present available, is metabolized in the body, largely by acetylation, to therapeutically inactive substances. Paraaminosalicylic acid (PAS) appears to compete with INH for the same acetylating enzymatic system. Thus simultaneous administration of both drugs results in higher serum concentrations of "active" INH. The present study was undertaken to investigate in infants and children the degree of INH inactivation, the effect of PAS on serum concentrations of "active" INH, the levels of "active" INH in the cerebrospinal fluid after oral administration, and the levels of INH in the gastric fluid. INH and PAS levels were determined according to a modification of the method described by Mayer, Whitney, Chambers, and Stanonis (Am. Rev. Tuberc. 76:852, 1957). The pattern of INH inactivation was studied in 14 children by determining "active" and "inactive"

INH at intervals of 1, 3, 12 and 24 hours after a standard test dose of oral INH (10 mg. per kilogram body weight). In 40 children serum levels of the two fractions were determined six hours after the test dose; this single determination gives a satisfactory clue to the speed of INH inactivation in a given individual. Our findings suggest that children closely resemble adults so far as INH metabolism is concerned. Thirty-eight of forty children given INH 20 mg. per kilogram per 24 hours together with NaPAS 0.5 gm. per kilogram per 24 hours exhibited serum concentrations of "active" INH of 0.4μg. per milliliter or more six hours after simultaneous oral administration of both drugs, this is generally accepted as the minimum "adequate" therapeutic level. The levels of "active" INH in the cerebrospinal fluid were many times greater than the bacterial level even 12 hours after an oral dose.

Concentrations of "free" and "total" INH in the gastric fluid of 50 children were determined at intervals of 12, 24, 36 and 48 hours after administration of an oral or intramuscular dose of INH (10 mg. per kilogram). "Active" INH in tuberculocidal amounts was present in most gastric fluid specimens aspirated 24 hours after administration of INH, and in some patients even after 48 hours.

70. A Preliminary Study of the Candida Albicans Hemagglutination Test and Its Potential Diagnostic Uses

NANCY HUANG,* Temple
Introduced by ROBERT H. HIGH.

A variety of bacterial antigens can be adsorbed onto red blood cells and such treated cells can then be agglutinated by homologous antibodies. The indirect test is highly sensitive for the detection of certain bacterial antibodies. We have recently employed this method for the detection of antibodies to Candida albicans in the sera of patients with moniliasis.

The soluble antigen which is adsorbed onto Type O human erythrocytes is prepared according to the method described by Vogel and Collins (Proc. Soc. Exper. Biol. & Med. 89:138, 1955). The following modifications of this method have been employed: the suspension of Candida albicans is shaken for three hours after which it is heated for one hour in a boiling water bath. The extracted antigen is concentrated four times before it is adsorbed onto red blood cells.

A total of sixty samples of serum obtained from infants and children with and without moniliasis have been tested. Patients with chronic moniliasis, such as one child with moniliasis and hypoparathyroidism and several in whom the disease is associated with leukemia have high titers of antibody against C. albicans. In acute attacks of moniliasis, however, the levels of antibody are quite variable and often no antibody is demonstrable.

The demonstration of a hemagglutinin response to C. albicans has many useful applications. It should be especially useful in determining the role of C. albicans in certain diseases such as acrodermatitis enteropathica, which has been attributed to chronic moniliasis even in the presence of positive isolations of the fungus. Other applications of this test will be discussed.

* By Invitation.

71. The Mechanism of Bacteriolysis by the Properdin System

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The properdin system, found in all normal mammalian sera, inactivates certain viruses and lyses various bacteria. It is presumably one of the mechanisms involved in natural resistance to infection. It has been shown previously (Wardlaw) that not all bacteria are susceptible to properdin. Most susceptible organisms are Gram-negative and belong to the Enterobacteriaceae. Susceptibility is a property of the strain, not species, genera, or tribe. The purpose of this paper is to present data relating to the properties of the bacterium which may determine this susceptibility.

1. Data obtained with Pillemer and Davies indicate that properdin interacts with the bacterial cell wall polysaccharide. Only those cell wall fractions containing polysaccharide combine with properdin. 2. Comparative studies with different bacterial strains suggest that two independent characteristics determine the differences in the susceptibility of the strains. The independence of these characteristics suggests that the rate of lysis is determined by more than the interaction of properdin and polysaccharide alone. 3. The rate of bacteriolysis is not determined only by the properdin concentration; the concentration of bacteria independently affects the process also. Thus one of the determining characteristics is in the bacterium itself. 4. Metabolically active and rapidly multiplying bacteria are more susceptible to lysis than resting organisms.

Thus bacterial characteristics influence the susceptibility of the organism to lysis. One characteristic is the bacterial cell wall polysaccharide, with which properdin interacts. Another characteristic is the metabolic activity of the organism.

These data reemphasize the importance of the nature of the parasite in the host-parasite relationship. The properdin system interacts with a variety of polysaccharide containing substances, derived from bacterial, mammalian, and other sources. Complex polysaccharides probably constitute the unifying substance which allows properdin to interact with such a diversity of infectious agents.

However, interaction of the bacterium with properdin does not alone determine the resultant lysis. The constitution and metabolic state of the organism also affect the response. Since the interacting polysaccharides are contained in the cell wall, and since interference with cell wall maintenance is the means by which certain antibiotics kill metabolically active cells, it is tempting to hypothesize that the properdin system has its effect by interfering with the ability of the organism to maintain an intact cell wall in perhaps an analogous manner.

72. Hemadsorption Viruses in Infections of the Respiratory Tract of Children

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Two new myxoviruses, hemadsorption viruses Types 1 and 2, have been isolated from children with respiratory disease by means of the hemadsorption technique in monkey kidney tissue culture. Type 2 is related to but distinct from Sendai virus. Type 1 virus was significantly associated with febrile illness during an outbreak of respiratory disease in a nursery. (Chanock et al., New England J. Med. 258:207, 1958).

This paper summarizes data on the relation of hemadsorption viruses to illness in children with severe upper and lower respiratory tract infection and in children seen in the clinic for less severe infection of the upper respiratory tract. In the first four months of this study hemadsorption viruses 1 or 2 were recovered from the oropharynx of (*a*) 7% of 382 infants and children with respiratory infections and 0.6% of 354 controls of comparable age; (*b*) 19% of 57 infants less than one year of age seen in the clinics with respiratory infection and none of 86 age-matched controls, and (*c*) 17% of 30 infants and children with a "croup" syndrome (Type 2 only).

Serological studies compiled to date show evidence of Type 2 hemadsorption infection in 32% of 22 children with a "croup" syndrome and in general confirm the other trends indicated by virus isolation.

These data suggest that; (*a*) hemadsorption viruses are etiologically related to infections of the respiratory tract in children; (*b*) they are most prevalent in infants under the age of one year, and (*c*) Type 2 hemadsorption virus may be associated with a "croup" syndrome.

* By Invitation.

73. Serologic Response of Children to Asian Influenza Vaccine

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Introduced by ROBERT B. LAWSON.

Normal children, mentally retarded children, and children with other physical and mental defects were immunized with Asian influenza vaccine containing 200 CCA units per milliliter. Injections consisted of 0.5 ml. of vaccine subcutaneously or 0.1 ml. intradermally. Some of the children were given repeated injections at 10 to 40 days following the first inoculation. Blood samples were taken at the time of initial inoculation, two weeks after the last injection, and sometimes at intervals in between. The serum specimens were subjected to complement-fixation and hemagglutination inhibition tests. The result showed that children responded to the vaccine as efficiently as did adults and, in some instances, much more efficiently. There was no significant difference between responses following intradermal injection of 20 CCA units intradermally and 100 CCA units subcutaneously. A second injection improved antibody response provided that it was given three weeks or later after the first inoculation. Relatively less boosting effect was noted when vaccine was reinjected at 10 days. The lack of an extensive epidemic in this area makes it impossible to draw final conclusions about the protective efficacy of the vaccine; however, the schools in which vaccine has been given escaped influenza whereas influenza outbreaks did occur in schools not receiving vaccine. This circumstantial bit of evidence may be regarded as at least an indication for the prophylactic value of vaccine. Recent findings in adults suggest that the protection afforded by the vaccine ceases at about three to four months after vaccination.

74. Severe Generalized Disease in the Newborn Due to Coxsackie Virus Group B

SIDNEY KIBRICK and KURT BENIRSCHKE,* Harvard

In the past few years it has become increasingly evident that infection with Group B Coxsackie viruses in the neonatal period may result in a severe and frequently fatal disease, characterized primarily by myocarditis but also with involvement of other organs.

Two additional fatal cases were studied. In each instance evidence indicated that the infection was acquired from the mother.

In the first a Group B Type 4 virus was present in the myocardium in high titer and also isolated from the feces of two siblings.

In the second, whose infection occurred in utero and who succumbed at 36 hours of age, Group B

Type 4 virus was recovered from the myocardium, spinal cord, and liver, as well as from the feces of the mother. No virus could be demonstrated in samples of the postmortem blood, lung, or psoas muscle.

The pathological lesions in these infants included myocarditis, meningoencephalitis, hepatic necrosis, pancreatitis, adrenal cortical necrosis and early fat necrosis. This widespread distribution of lesions is analogous to the results of experimental infection with certain Group B virus strains in the suckling mouse.

An additional feature of interest with possible bearing on the role of these agents in paralytic disease in man was the nature of the central nervous system lesions induced in newborn mice by each of the recovered viruses. This consisted of an acute destruction of the anterior horns of the spinal cord indistinguishable from that induced by poliovirus.

An analysis of 25 cases of this disorder revealed that the most prominent clinical features were fever, feeding difficulties, lethargy, and signs of cardiac or respiratory involvement. In all of the sporadic cases on which data were available the mother had experienced a febrile or respiratory illness shortly before her infant became ill.

75. Disease in Monkeys Inoculated with ECHO Virus

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Introduced by EDWARD C. CURNEN.

In previous reports certain ECHO viruses have been implicated as causative agents of neurologic disease in man. In order to determine whether viruses of this group could produce paralysis and neuronal damage in monkeys the following experiments were carried out.

Strains of ECHO virus type 6 (Shobakan) and type 16 (Harrington) were each injected intrathecally into Rhesus or Cynomologous monkeys. Of the five animals injected with each agent four received active virus propagated in human amnion cells, and the fifth received the same material heated at 65 C for 30 minutes.

The animals were examined daily for evidence of disease or weakness. Every other day specimens of blood and CSF were collected for determination of the leucocyte count and the presence of virus. During the period of 21 days after injection, individual monkeys were sacrificed and specimens of the CNS were examined histologically.

All of the animals which received active virus developed leucocytosis of the CSF whereas those which received heated material did not. Six monkeys developed weakness of one or more extremities, four had demonstrable viremia, and in seven virus was recovered from the CSF. At autopsy, microscopic examination of the spinal cords revealed meningeal infiltration of round cells. Definite neuronal changes, however, were not evident.

76. Alteration of Antigenicity of Poliovirus

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Columbia
Introduced by HATTIE E. ALEXANDER.

Sporadic reports have appeared in the literature suggesting that Type 1 and 2 poliovirus may have a common antigen on the basis of serological "crossing" in complement-fixation tests and possible cross protection against one type following infection with the other type in monkey experiments. This paper will present further evidence in favor of a common antigen in that the antigenic specificity of both Types 1 and 2 poliovirus has been altered reproducibly by repeatedly freezing and thawing the same virus sample. The resultant virus can be neutralized by either Type 1 or Type 2 antibody.

The altered specificity was first detected when a single aliquot of Type 2 virus was repeatedly frozen and thawed over a period of several weeks while being used as a control suspension in neutralization experiments. The Type 2 virus which was uninhibited by the presence of Type 1 antiserum throughout most of this interval suddenly became neutralizable by Type 1 as well as by Type 2 antiserum. The change was consistently demonstrable in this sample and was accompanied by a significant drop in titer. New populations of virus obtained by passage of this altered material did not show this changed specificity. The change is apparently phenotypic, not genotypic.

Subsequently similar antigenic alteration has been produced in standard Types 1 and 2 polioviruses whenever the attempt has been made to produce the change. Relatively slow freezing and thawing (electric freezer temperature and room temperature) as opposed to "rapid" freezing and thawing (dry ice and alcohol and 37 C water bath) has proved to be a much more efficient method of producing the change.

The Dulbecco technique in HeLa cell monolayers, which permits an accurate measure of neutralization of virus, was used as the tool in these experiments.

77. The Relationship of Identifiable Viruses and the Clinical Pictures of Poliomyelitis and Aseptic Meningitis

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Introduced by BRUCE D. GRAHAM.

In 1956 and 1957, there were admitted to the Contagious Disease Ward of the University Hos-

* By Invitation.

pital 146 patients with a diagnosis of poliomyelitis or "aseptic meningitis." In 101, at least two attempts to isolate virus from the stool, throat washings, or C. S. F. were made and two sera obtained for neutralizing poliomyelitis antibodies. Eighteen patients had fewer studies but enough to permit agent identification.

Clinically 52 patients had some paralysis that persisted beyond the acute illness. Sixty-seven patients had an acute febrile illness with meningeal irritation, cerebrospinal fluid changes but no paralysis, consistent with the diagnosis of nonparalytic poliomyelitis or aseptic meningitis.

Of the 49 paralyzed patients, 48 had laboratory evidence of poliomyelitis; 29 with both isolation of virus and development of neutralizing antibodies; 14 by antibody titer changes (four fold increase) or isolation and identification of the virus. Three patients excluded from the study because of inadequate number of isolation attempts were paralyzed and had no rise in antibody titer against poliomyelitis virus.

From the 71 nonparalytic patients a diagnostic antibody titer change or virus isolation was obtained in 32. Four of these were poliomyelitis virus, 11 were ECHO-9, and 17 were Coxsackie viruses (A-9, B-3, 4, and 5). In 39 no agent was identified. The very small number of nonparalytic patients exhibiting laboratory evidence of poliomyelitis seems impressive to us.

As is well known the presence of other enteric viruses makes the isolation of poliomyelitis virus more difficult. In view of this it is interesting that three cases of dual infection were observed.

The clinical pictures associated with the various virus agents will be described and contrasted.

78. A Clinical, Epidemiologic, and Laboratory Study of Illness Due to ECHO-9 Virus in a Housing Development

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Introduced by FREDERICK C. ROBBINS.

During the summer of 1957, an extensive outbreak of a febrile disease associated in some cases with a rash occurred in Milwaukee, Wisconsin. Simultaneously there was an increase in the incidence of aseptic meningitis which was occasionally accompanied by a rash. Accordingly, a study of the disease was conducted in a population of 320 families living in a suburban housing development. We wish to present data concerning clinical features of the disease, excretion of virus by patients, and distribution of virus in this community.

Headache, rash, fever, and nuchal rigidity were considered to be the major characteristics of the disease. Symptoms and signs varied according to age, the rash appearing predominantly in younger children, fever and headache in older children, and meningeal irritation in the adults. The rash was red, fine, papular, and nonpruritic. It usually occurred on the face and trunk but in some instances the entire body was involved, including palms and soles. Respiratory symptoms, lymphadenopathy, photophobia, and conjunctivitis were absent. The rash usually appeared within 24 hours after the onset of fever and lasted from 1 to 10 days. Recurrences of rash and fever were observed in several individuals after asymptomatic periods.

The attack rate as determined by clinical manifestations was 28%. The incidence of illness in children under 9 years of age was twice that of persons over 19 years. The familial incidence of this disease was striking.

Fecal specimens and paired sera were obtained from 97 families for laboratory study. In 42, at least 1 individual manifested signs characteristic of this disease. The remainder had no illness. Monkey kidney cells were used for virus isolations from fecal specimens. With few exceptions the isolates were identified as ECHO-9 virus. Although suckling mice were unsatisfactory for primary isolation of ECHO-9 from fecal specimens, infected tissue culture fluids were found to produce disease characteristic of Group A Coxsackie viruses. Serologic studies are in progress to extend the laboratory analysis of this illness.

An attempt was made to correlate clinical illness with viral excretion. In 75% of the families experiencing illness, at least one individual was found to be excreting virus if the specimens were obtained within 10 days after onset of illness in the household. In contrast, virus was found in only 24% of the households without illness. The recovery rate of virus among sick individuals was 75% for children under 4 years of age, and 33% for persons over 19 years. In most instances, virus excretion persisted for less than three weeks after the onset of illness.

We believe that the evidence presented here, as well as isolation of ECHO-9 virus from cerebrospinal fluid specimens from hospitalized cases, indicates a wide spectrum of disease due to one etiologic agent.

79. Isolation of Virus of Measles from Three Fatal Cases of Giant-Cell Pneumonia

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Harvard
Introduced by SIDNEY KIBRICK.

Giant-cell pneumonia is a condition characterized clinically by pneumonitis and pathologically by the presence of multinucleated giant cells with intracytoplasmic and intranuclear inclusion bodies in

* By Invitation.

the pulmonary lesions. So far the etiology of this condition has remained obscure.

In this report isolation of an agent serologically and cytopathogenically indistinguishable from the virus of measles from three fatal cases of giant cell-pneumonia will be discussed.

Three children suffering respectively from mucoviscidosis, leukemia, and Letterer-Siwe disease died with respiratory symptoms. The diagnosis of giant-cell pneumonia was made on postmortem examination. None of these children had a rash or any other signs of measles during the terminal phase. None had a previous history of measles, but all of them had been in contact with rubeola.

Lung tissue from all three cases and liver from one of them were available for viral studies. Human kidney tissue cultures were inoculated with 10% suspensions of these specimens. Multinucleated giant cells were observed in tissue culture after five to seven days of incubation. In stained preparations intranuclear and intracytoplasmic inclusion bodies were conspicuous. These changes were indistinguishable from those produced by the virus of measles. The cytopathogenic effects of the isolated agent were prevented by convalescent human and monkey measles sera. Cross neutralization was demonstrated between measles virus and the convalescent serum of a monkey infected with the agent isolated from the giant cell pneumonia cases. The virus was not neutralized by convalescent dog distemper serum. Complement fixation tests with these sera confirmed the results of the neutralization tests.

In addition the following experiments were conducted to define the characteristics of the isolated agent. 1. The agent was passed without difficulty in the human kidney tissue culture, and was adapted to human amnion, monkey kidney, and HeLa tissue cultures. 2. A monkey was inoculated with the agent of giant-cell pneumonia. Virus was recovered from the blood of the animal five days after inoculation which caused typical cytopathogenic changes in the tissue cultures. A significant antibody rise was demonstrated in the monkey's blood. 3. The agent did not multiply in the chorioalantois, the amnion, or the yolk sack of the chick embryo. 4. Suckling mice inoculated intraperitoneally and intracerebrally with the virus survived without symptoms.

The agent isolated from cases of giant-cell pneumonia appears to be identical with that of measles. The clinical picture, however, differs from classical rubeola. The possibility exists that this difference is due to altered host-virus relationship. The three children from whom the virus was recovered were suffering from chronic debilitating diseases, which might have altered the response to the measles infection.

80. Glutathione-Sensitive Chronic Nonspherocytic Hemolytic Anemia

W. A. NEWTON JR.* and J. C. BASS,* Ohio State
Introduced by WARREN E. WHEELER.

A four-year-old Italian boy was hospitalized because of an acute episode of anemia and hemoglobinuria. He had been given Gantrisin for a fever and cough for two days, started passing dark urine, and was admitted three days later. His red cells were Coomb's negative, normocytic, normochromic, showed occasional schizocytes, and did not have an increased osmotic fragility. No Heinz bodies were seen. The diagnosis of Gantrisin-induced acute hemolytic anemia, with hemoglobinuria was made.

This child, in the neonatal period, had developed unexplained hyperbilirubinemia, and had frequently been "sallow" since that time. He had had one other episode of dark urine following an unknown medication a few years previously.

He was treated symptomatically, without blood transfusion, and showed prompt cessation of the hemoglobinuria and a rapid rise in his hemoglobin concentration. He was discharged home after six days hospitalization, and he was followed as an outpatient. He has not shown any recurrence of the gross hemoglobinuria, but has continued to have reticulocytosis, in the range of 4.6% to 13.3%. Although his urine has been yellow in color, it has tested positive for blood in the absence of microscopic hematuria.

Twelve weeks after the acute hemolytic episode, the red cell reduced glutathione concentration, before and after incubation with acetylphenylhydrazine and Gantrisin, was performed on the red cells of the child, his parents, and his brother and sister. The family showed no significant change. The child's fresh red cells showed GSH concentration of 45 mg/100 ml. of red cells. After incubation with acetylphenylhydrazine and Gantrisin, it was 11 mg. % and 29 mg. %, respectively.

Because of these findings, red cell survival studies were performed on the cells of the child and the parents. The half-life of the child's cells was five days, but both parents' cells showed no shortening of their life span by this technique. Determination of the GSH levels of the child's red cells following acetylphenylhydrazine in the presence of glucose and the concentration of glucose-6-phosphate dehydrogenase will be performed.

While it appeared that this child had a drug sensitive red cell defect, similar to that described by Beutler, Zinkham, and others, he is different in at least two respects.

First, his red cell defect appears to be congenital in origin, manifesting itself in the absence of, but accentuated by, ingestion of Gantrisin.

* By Invitation.

Secondly, we have not been able to demonstrate a similar defect in either of his parents and siblings. This is in contrast to the sex-linked dominant pattern of inheritance of the asymptomatic drug-sensitive person described by others.

This appears to be an example of a congenital, nonspherocytic red cell defect, manifesting itself as a compensated chronic hemolytic process, which is accentuated by ingestion of Gantrisin.

81. Observations on the Glutathione (GSH) Stability of Red Blood Cells

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Harvard
Introduced by John D. Crawford.

Glucose-6-phosphate (G-6-P) dehydrogenase deficiency and erythrocyte glutathione (GSH) instability have been described by Zinkham as occurring in Negroes with naphthalene poisoning. At his suggestion, a study of these factors in a Caucasian child with a past history of hemolytic anemia following naphthalene ingestion was carried out. Both erythrocyte GSH instability and G-6-P dehydrogenase deficiency were found in the patient, although neither the parents nor the siblings exhibited either of these abnormalities. The addition of a complete reduced triphosphate pyridine nucleotide (TPNH) generating system (TPN, glucose-6-phosphate, and G-6-P dehydrogenase) to the patient's hemolysate resulted in the reversal of the GSH instability.

In view of these findings, a survey of GSH stability in Caucasians with deficiencies of other enzymes involved in carbohydrate metabolism, with other types of hemolytic anemia and with chemical poisonings was carried out. No evidence of GSH instability was found in galactosemia, glycogen storage disease, congenital hemolytic anemia, acquired hemolytic anemia of the extracorpuscular type, the hemolytic anemia of erythroblastosis fetalis, lead poisoning anemia, iron deficiency anemia, or agranulocytosis due to mesantoin intoxication. However, transitory erythrocyte GSH instability was observed in some patients with advanced liver disease, who had recently received massive transfusions during ligation of bleeding esophageal varices. Normal G-6-P dehydrogenase was found in these patients and the addition of a complete TPNH generating system to the hemolysate reversed the GSH instability.

These observations suggested that the phenomenon of GSH instability was corrected by a single method, although produced through different mechanisms. The observation by Beutler that GSH instability, without an accompanying G-6-P dehydrogenase deficiency, occurred in blood after standing at room temperature, suggested that the erythrocyte GSH instability in the transfused patients may have been due to the transfused erythrocytes whose GSH had become unstable during storage. To examine this possibility further, the levels and stability of erythrocyte GSH in bank blood were studied. The effects of collection in Acid-Citrate-Dextrose, ethylenediaminetetra-acetate, and over an ion exchange column were compared and it was found that the level of GSH declined steadily during storage. The instability of GSH was found in only a few samples of ACD blood late in the third week, but earlier (at 14 days) in EDTA and ion exchange blood. It was noted that this GSH instability could be corrected by the addition of adenosine.

It is apparent from this study that GSH instability is not confined to a single racial group. It may occur in the absence of specific drugs (e. g., naphthalene) and without a demonstrable deficiency of G-6-P dehydrogenase. Moreover, the loss of important metabolites from the cell may contribute to this instability, as it develops in stored blood.

82. The Antibody Elution Test in Heterospecific Pregnancies

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The study of hemolytic disease resulting from incompatibilities in the ABO system has been continued with particular reference to serologic methods designed to differentiate between sensitized and nonsensitized infants. Attempts have been made to elute antibody from infants' red blood cells at 56 C. This may be done if (1) Fresh blood is used. (2) The infants' cells are refrigerated constantly until the elution is performed. (3) The eluted antibody is sought in colloidal media such as 10% acacia or by means of the antihuman globulin (Coombs) technique.

Elutions have been performed on blood from 89 infants. An attempt has been made to correlate the results of these studies with those obtained by the "glue test" and with the presence of icterus. Positive elutions were obtained from the cells of 10 infants, only 3 of whom were icteric. Of those infants from whose cells antibody was eluted, only three showed a positive glue test. Neither the glue test nor the elution test can be correlated with the presence of icterus since only 3 of 47 icteric infants had a positive elution and only 5 had a positive glue test.

Three infants who became jaundiced in the first 24 hours of life and required exchange transfusions had strongly positive glue tests and antibody elution tests. Because of the frequency with which antibody may be eluted from the red blood cells of normal infants, however, this test is unsatisfactory

* By Invitation

for the detection of hemolytic disease due to incompatibility in the ABO system.

83. Sequestration of Iron in the Lungs in Idiopathic Pulmonary Hemosiderosis

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Introduced by HOWARD A. JOOS.

A female child was followed clinically for three years with the diagnosis of idiopathic pulmonary hemosiderosis. She manifested a chronic hypochromic microcytic anemia, decreased serum iron and had no stainable iron demonstrable in bone marrow, skin biopsy, or urinary sediment. Sputum contained large number of cells containing multiple granules stainable by the Prussian blue reaction. At autopsy various tissues were analyzed for their iron content with the finding that hemosiderosis existed only in pulmonary tissue, whereas other tissues had low concentrations of iron.

Tissues from another case of idiopathic pulmonary hemosiderosis were analyzed and revealed similar findings. For comparison, two cases of chronic heart failure, two cases of transfusion hemosiderosis and two previously well children dying accidentally were studied.

Results are tabulated as follows.

The total quantity of iron per organ is influenced by the variation in organ weights due to differences in age; however, the six-year-old child had over 4 gm. of iron in pulmonary tissue while the 13-year-old had approximately 2.5 gm. The cases with chronic heart failure revealed 0.2 and 0.8 gm. in the lungs, but they had no evidence of increased iron content in other tissues.

The patients with transfusion hemosiderosis had 7.8 and 12.9 gm. of iron in their livers. One of these, a patient with chronic hemolytic anemia also had over 3 gm. in the spleen. The other, with aplastic anemia, showed only 0.15 gm. None of the organs of the normal children contained as much as 0.3 gm.

These data document the selective deposition of iron only in the lungs in idiopathic pulmonary hemosiderosis and illustrate moderate deposition in lungs in chronic heart failure. The deposition of iron in transfusion hemosiderosis is primarily in the liver and to a lesser extent in the spleen.

The presence of very large quantities of iron in the lungs in idiopathic pulmonary hemosiderosis was accompanied by the clinical and laboratory findings of chronic iron deficiency anemia. This paradox suggests that the pulmonary hemosiderin deposits are sequestered from the metabolic pathways and are not physiologically available for hemoglobin formation.

* By Invitation

Diagnosis	Age	Lungs *	Liver *	Spleen *
Pulmonary hemosiderosis	6	420	85	10
Pulmonary hemosiderosis	13	136	28	38
Heart failure	15	88	29	56
Heart failure	45	50	17	40
Transfusion hemosiderosis	13	46	80	103
Transfusion hemosiderosis	39	42	284	285
Normal	8	24	39	45
Normal	10	22	32	26

* Measurements in mg. of Iron per 100 gm. wet tissue.

84. Alteration in the Electrophoretic Characteristics of Hemoglobin in "Paint Eaters"

A. H. TUTTLE and CHARLES FITCH,* Tennessee

The hemoglobin of two children with a history of pica (paint eating) has been found to consist of two fractions. The major component is electrophoretically similar to Hgb A (paper electrophoresis, pH 8.6) while the minor component exhibits a more rapid mobility than Hgb A and separates from the major component incompletely even after prolonged electrophoresis. One of the patients was found to have evidence of heavy metal deposition in the long bones which disappeared gradually during a year's period of observation. During this time his hemoglobin became electrophoretically normal. The second patient expired shortly after our initial examination of his hemoglobin with hematologic and neurologic signs usually associated with severe lead intoxication. We attribute this in vivo alteration of hemoglobin to the effects of a component of paint, presumably lead. It has not been possible to produce a similar alteration in vitro by lead nor by producing chronic lead poisoning in experimental animals.

85. Thalassemia-like Syndromes Due to Recently Discovered Abnormal Hemoglobins

PARK S. GERALD* and LOUIS K. DIAMOND, Harvard

In a survey of hemolytic anemias, two of the more recently discovered abnormal hemoglobins (Lepore hemoglobin and Hgb H) were found to be etiologic factors in the production of thalassemia-like syndromes. Of especial significance in the differentiation of these conditions from "classical" thalassemia was the inability of paper electrophoresis to demonstrate the abnormal hemoglobin, although both were detectable by starch block electrophoresis.

The Lepore hemoglobin was encountered during the investigation of the family of an infant with clinical thalassemia major. The propositus ex-

hibited all the features expected in thalassemia major; onset in early infancy of a severe hemolytic anemia associated with bizarrely shaped erythrocytes, normoblastemia, marked hepatosplenomegaly, Italian ancestry, and high levels of fetal hemoglobin. The patient's father exhibited the typical morphologic and electrophoretic (increase Hgb A_2) findings associated with thalassemia trait. Although the mother presented the same morphologic features as the father, her hemoglobin electrophoretic pattern was unique in possessing an abnormal hemoglobin (the Lepore hemoglobin). From physicochemical studies of her hemoglobin, it is apparent that the abnormal component differs from any of the previously described hemoglobins. Four other instances of the abnormal hemoglobin were encountered among the mother's relatives and in each case the erythrocyte morphology was similar to that found in thalassemia trait.

The second group of thalassemia-like diseases due to an abnormal hemoglobin is typified by the case of an Italian woman with the clinical appearance of thalassemia minor (the Rietti-Greppi-Micheli syndrome) associated with the presence of trace amounts of Hgb H. Of two children born to this woman, one was found to have Hgb H in the cord blood and a mild alteration in the morphology of the red cells. The Hgb H in this newborn was likewise present in only trace amounts. This syndrome may account for some of the thalassemia variants reported by Italian authors in which no abnormal hemoglobin appeared on paper electrophoresis.

86. Studies in Sickle-Cell Anemia: Further Studies on Hepatic Function

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There is considerable clinical and pathological evidence of hepatic involvement in sickle cell anemia (SCA). This paper is an attempt to sample the extent of altered hepatic function by the application of the following tests: traditional liver function tests, serum protein electrophoresis, determination of the levels of venous blood ammonia, analysis of amino acid excretion of the urine, and histological examination of liver tissue.

Forty-nine admissions of 21 children with SCA ranging in age from 2 to 13 years were used in one portion of the study. Studies included icterus index, serum bilirubin, Van den Bergh, cephalin flocculation, alkaline phosphatase, total proteins, A/G ratio, cholesterol, serum glutamic oxaloacetic transaminase, and urobilinogen in the urine and feces. Of the total number of admissions, 25 or 51% were found to have an enlarged liver and 21 or 43% showed clinical icterus of variable degree. Examination of the data obtained from traditional liver function studies gave only suggestive evidence of hepatic damage.

Serum protein separation by filter paper electrophoresis was carried out on 28 children with SCA ranging in age from 10 months to 16 years. Fourteen of the children exhibited an elevation in the γ-globulin fraction, and four showed an elevation in the β-globulin fraction.

Venous blood ammonia levels were determined (modification of the method of Seligson and Seligson using photoelectric colorimetry) on 24 known SCA patients and 21 nonanemic children (controls). In 28 determinations obtained during a quiescent period on 24 children, the value ranged from 21 to 104μg. per 100 ml. A value of 127μg. per 100 ml. was obtained on one patient in crisis with an associated pneumonia. Four other patients with SCA who were in crises associated with mild respiratory infections showed values ranging from 20 to 97μg. per 100 ml. The results obtained on the 21 children used as controls were 23.0 to 85.0μg. per 100 ml. The data obtained from patients were generally within the normal range, regardless of whether they were examined during an asymptomatic period or during a crisis.

Two dimensional paper chromatography was used to separate and identify the amino acids in the urine of five children with SCA. One child was studied during a crisis associated with an infection. No unusual or abnormal amino acids were found in the urine of the patients observed thus far. Quantitative studies are incomplete.

A liver biopsy specimen obtained on a five-year-old male with SCA in crisis with pneumonia showed the sinusoids to be greatly dilated and plugged with sickled cells. In addition, there were areas of degeneration of the cord cells. It is noteworthy that the routine laboratory tests for liver function in this case gave only suggestive evidence of hepatic dysfunction.

In young children, the correlation between liver damage and the results or response obtained from traditional function tests is less reliable than in older individuals. In children with SCA, histological examination of tissue obtained from liver biopsy affords the most reliable information concerning the status of hepatic damage.

87. Studies of Kernicterus in Genetically Jaundiced Rats

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Gunn described a strain of rats with an inherited form of jaundice. Others have shown the defect to be the result of a deficiency of glucuronyl transferase in the liver. We have found that kernicterus develops in these rats that is indis-

* By Invitation

tinguishable from the kernicterus of human beings. It is our purpose to describe the natural history of the disease in these rats and the effect upon it of hypoxia, sulfa drugs, and administered glucuronic acid.

Sixty-five per cent of 135 jaundiced rats of this strain developed kernicterus during the first month after birth. Half of the jaundiced survivors had motor defects as adults. Twenty-seven rats with kernicterus demonstrated at postmortem had serum indirect bilirubin concentrations averaging 17 mg/100 ml. shortly before death. In contrast, 41 which survived had peak bilirubin levels which averaged 11.5 mg/100 ml. The highest level we have measured in a young jaundiced animal which survived to adulthood was 16.5 mg/100 ml. This animal had mild ataxia.

The newborn period is characterized by an intensification of the jaundice. The anemia which also develops during the newborn period is not greater than that seen in normal nonjaundiced rats.

Signs of kernicterus, if they are to appear, almost always develop during the first three weeks of life. One interesting exception was noted in a rat which first developed motor dysfunction at four months, at a time of unexplained transitory elevation of serum bilirubin above the usual adult level of 6.3 mg. to 18.8 mg. per 100 ml. The disease in this rat presents parallels with that in Rosenthal's jaundiced patient who developed central nervous system abnormality at three years. Other adult rats have successfully withstood similar transitory episodes of intensified jaundice.

Silverman observed kernicterus in young premature infants treated with sulfasoxazole, despite only moderately elevated levels of serum bilirubin. We found that therapeutic doses of that drug and sulfadiazine lowered the bilirubin levels of young rats by 30%. Only 1 of 35 so treated survived, and at postmortem especially intense staining of brain and fatty tissues was seen. Conceivably the drugs had altered membrane permeability. Adult jaundiced rats were not adversely affected by these drugs.

Holt has reported the efficacy of administered glucuronic acid in the treatment of jaundiced infants. The serum bilirubin of 20 adult rats was not altered by similar treatment, but that of 5 young rats was lowered by injections of sodium glucuronate. Kernicterus was not prevented; in fact, it was somewhat intensified.

Forty-nine jaundiced and 127 nonjaundiced rats were exposed to an atmosphere of 100% nitrogen once a day for seven days after birth. Surprisingly, 23% of the control and only 10% of the jaundiced rats died. The survivors had no increase in incidence of kernicterus.

The enzyme-deficient young of jaundiced dams are not jaundiced at birth. Bilirubin levels confirm this observation. In eight young from two litters, bilirubin determinations at two hours of age showed levels only 18% to 50% as high as those of the mothers. Since bilirubin rises rapidly after birth, the contrast between fetal and maternal blood must be greater than these two hour figures would indicate.

Papers Read by Title

88. Cause of Anemia in Erythroblastosis Fetalis

Fred H. Allen Jr.; Louis K. Diamond, and John M. Craig, Harvard

New data strengthen the theory that the anemia of erythroblastosis fetalis is, in many cases at least, the result of deficiency of one or more essential factors. Infants dying of erythroblastosis were classified by histologic examination of the liver into four groups depending on the degree of maturity of the red cells in the foci of hematopoiesis. In some cases the majority of hematopoietic foci consisted of very mature forms, while in others most foci consisted of nearly mature erythrocytes. The pathologist's evaluation was then compared with the calculations of actual blood formation based on reticulocyte and nucleated red cell counts and hemoglobin concentration. In most cases where the red cells appeared to be most immature, indicating presumably the greatest "effort" on the part of the baby, the actual red cell production was low. This is presumed to indicate maturation arrest. Otherwise, production should have been greater in this group, rather than less. On the basis of these data, as well as previously reported data, it is postulated that anemia in erythroblastosis fetalis is the result not only of abnormally rapid red cell destruction, but of simultaneous failure of adequate red cell production due to relative insufficiency of essential substances.

89. The Effects of Vascular Distention on Inflation of the Newborn Lung

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Introduced by Clement A. Smith.

It has been postulated that acute distention of the pulmonary vascular bed, occurring at birth, has an erectile effect on the lungs and is a primary event in the first breath. This hypothesis rests upon evidence in part from the histological appearance of the lung after vascular injection, and in part from the recorded volume change of the lung before the first breath. The present investigations were designed to measure directly the forces created in the lung by distention of the vascular bed.

* By Invitation

Gas-free lungs of cats and dogs and the lungs of two human stillborn infants were studied in the excised state. By cannulating the pulmonary artery and the left auricle, isolated from the rest of the heart, and filling the system with Dextran in Ringer's-lactate, it was possible to elevate the pressure in the pulmonary vascular bed to known levels, and at the same time record the volume changes in this circuit. An intratracheal cannula was used to record simultaneous pressure changes in the lung with respect to the atmosphere. In the gas-free lungs of adult animals, at a vascular pressure of 20 to 30 cm. H_2O, the pressure in the lung was only 1 to 1.5 cm. below atmospheric. At vascular pressures of 70 cm. H_2O, the pressure in the lung was 5 cm. below atmospheric. At this level of vascular distention, however, edema was formed after a few seconds, elevating the pressure in the lung from the initial —5 cm. to slightly positive pressures. In the lungs of the human stillborns, which contained some amniotic fluid, the effect of vascular distention on the pressure in the lung was negligible.

In order to determine the significance of the force created by vascular distention, additional experiments were done to measure the pressures required to inflate these lungs when they were totally collapsed and when they contained known amounts of fluid. Opening pressures were 20 to 25 cm. H_2O in the collapsed lungs, and 15 to 20 cm. H_2O in the lungs containing some fluid. Thus the effect of fluid in the lung is to facilitate opening. On the other hand, as shown earlier, fluid would negate the effect of vascular distention. In no instance would the pressures exerted by capillary distention alone approximate the pressures required for inflation.

90. The Use of a Magnetic Tape Recorder in Electromyography

HENRY W. BAIRD III, Temple

Electromyographic tracings have been recorded on magnetic tape and played back through the writing system of a standard Grass EEG machine. Because the time-base and the amplitude of the muscle potentials can be altered as the tracing is played back, slight changes can be more readily detected. The apparatus consists of an Ampex FR-100 4 channel tape recorder, a specially constructed amplifier, and a standard Grass EEG machine. The system, which amounts to a 4 channel ink-writing oscilloscope, has a greater frequency response than the standard EEG machine. It has also been used for recording electroencephalograms and subcortical electrograms. The results thus far suggest that various relaxing and tranquilizing agents alter muscle tone but do not improve functional ability.

91. Studies on Adenovirus Infections in Infants and Children

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During a six month period beginning January, 1957, adenovirus isolations were attempted from nose and throat, and, in some instances, rectal swabs taken from children seen in the clinic or hospitalized with acute respiratory infections. Similar procedures were carried out on a group of well children and children with various other acute and chronic illnesses.

A total of 506 children were studied. 324 of these had acute respiratory illness, 47 had Group A streptococcal infections, 54 had other acute and chronic illnesses, and 81 were well.

Twenty-six adenovirus isolations were made from the total group: 20 from the acute respiratory infection group, 1 from the group with streptococcal infection, 2 from children with other illnesses, and 3 from well children.

There appeared to be a relation between age and rate of virus isolation. In children with acute respiratory infections the isolation rate from those less than two years of age was 10% as compared to 5% from those over two years of age. All three isolations from well children were from infants less than six months of age.

Adenoviruses of Types 1, 2, 3, 4, and 5 were isolated. The majority of the isolations were of types 1 and 2. In both the 0 to 2 year and 2 to 4 year age groups, ⅔ of the isolations were of these types. Of the nine Type 1 isolations, five were in the 0 to 6 month age group and five of the six adenoviruses isolated in this age group were Type 1.

Only one Type 4 adenovirus was isolated, and this was from the child of a man in military service, who resided in the town adjoining his father's station. Of the children with acute respiratory infections, diffuse involvement of the respiratory tract with productive cough was the most frequent clinical picture seen; croup or, in older children, hoarseness was present in about ¼ of the cases. Two of the children had pneumonia.

Serologic studies were available on only a portion of this group. In most of these instances there was a convalescent rise in the neutralizing antibody titer to the type adenovirus isolated.

This work extends the observations of others on the prevalence of adenoviruses in well children and those with acute respiratory diseases.

* By Invitation

92. Glycogen Synthesis and the Mechanism of Action of Insulin

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The current theory of the mechanism of action of insulin is based on an apparent effect of this hormone on the entrance of glucose into the cell. The diabetic cell is presumed to be starved of glucose due to the patient's lack of insulin.

It is possible to show that the diaphragm muscle of the rat becomes permeable to xylose under the influence of insulin. The anatomic structure of the diaphragm makes it impossible to cut the muscle from its attachments without cutting almost every cell in the tissue, rendering it less impermeable to xylose in the absence of insulin than an intact diaphragm.

Glycogen synthesis, if the current theory is correct, should depend upon the same factors which govern xylose permeability, since both xylose and glucose have been shown to move into the cell under the influence of insulin. By cutting rat diaphragm so that all cells are broken it is now possible to show that glycogen synthesis does not occur without insulin under conditions which permit free diffusion of sugars into the cell. In diabetes, therefore, the limiting factor in glycogen synthesis is not the glucose content of the cell, but apparently insulin has a more direct metabolic effect than merely the movement of sugars into the tissues. This experimental work has been done with rat diaphragms and also with a new preparation of pigeon breast muscle which will be described.

93. Avitaminosis E in Cystic Fibrosis of the Pancreas: A Morphologic Study of Gastrointestinal and Striated Muscle

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Deposition of ceroid-pigment in smooth muscle and other tissues and degenerative changes in striated muscle are common findings in experimental avitaminosis E. Low tocopherol levels in the blood of patients with cystic fibrosis of the pancreas (CFP) and biliary atresia have recently been demonstrated. The present study was undertaken to determine the age of onset, frequency and severity of the changes of smooth and striated muscle in CFP and to compare them to those observed in other conditions.

A search for ceroid-pigment deposits in the gastrointestinal tract was made in 150 cases of CFP. In 82 cases, sections from esophagus, appendix and ileum were available. The amount of pigment was graded and compared to the age of the patients. A similar search was made in cases of biliary atresia, celiac disease, liver cirrhosis, and controls. One-hundred-eighty-five sections of muscle in CFP were reviewed. In 15 cases, 6 to 10 muscles were examined.

A diffuse deposition of ceroid-pigment was found consistently in the smooth muscle layers of the gastrointestinal tract of older patients with CFP. The deposition began in the second year and was frequently extensive in the fourth year of life. Similar changes were observed in 18 cases of biliary atresia and appeared to occur earlier in this condition than in cystic fibrosis of the pancreas. No ceroid was found in control cases.

Only two instances of changes of the striated muscle have been found in CFP. These changes do not appear to be part of a generalized process. They bear no relation to age, severity of the disease, and amount of ceroid deposit in the smooth muscle.

Extensive ceroid deposit probably represents an expression of prolonged and severe vitamin E deficiency related to protracted malabsorption of fat-soluble tocopherols. A beneficial effect of vitamin E supplementation is yet to be proven. However, because of the demonstration of low blood levels of tocopherol and because of the morphologic evidence reported here, it would seem logical to include vitamin E in the therapeutic regimen of cystic fibrosis of the pancreas.

94. The Possible Mechanism and Site of Bilirubin Inhibition of Electron Transport

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An enzyme preparation of beef heart mitochondria as described by Crane, Glenn, and Green was used. Cytochrome-c-reductase, DPNH oxidase, and succinate oxidase were tested according to the method of Lehman and Nason.

There was inhibition of cytochrome-c-reductase and succinate oxidase. DPNH oxidase was unaffected. The inhibition of cytochrome-c-reductase and succinate oxidase was checked in the Warburg in terms of oxygen consumption as well as in the spectrophotometer. This inhibition ranged from 25% to 100% depending on the final concentration of indirect bilirubin 2 mg. % to 20 mg. %. In a similar concentration biliverdin showed no inhibition.

When the bilirubin inhibition cuvettes were then scanned at various wave lengths it was noted that the bilirubin had been converted to mesobilirubin, which is interpreted as meaning the bilirubin is acting as a hydrogen acceptor.

Spectrographic analysis of bilirubin in the cytochrome-c-reductase and succinate oxidase runs demonstrated that the bilirubin wave length had shifted from 450 to 422.

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*Cytochrome-c-reductase and Succinate Oxidase Runs: D. U. Spectrophotometric Readings at 550 (Reduced Cytochrome-c)**

	Time						
	0 Min.	½ Min.	1 Min.	1½ Min.	2 Min.	2½ Min.	3 Min.
Control	0	.275	.400	.500	.520	.520	.520
Bilirubin 0.7μM	0	.000	.000	.000	.000	.000	.000
Bilirubin 0.07μM	0	.140	.245	.295	.300	.300	.300
Biliverdin 0.7μM	0	.275	.400	.500	.520	.520	.520

DPNH Oxidase Run
D. U. Spectrophotometric Readings at 340 (Oxidized DPN)*

Control	1.000 (1 sec.)
Bilirubin .7μM	1.000 (1 sec.)

Warburg Runs with DPNH and Succinate as Substrates

	DPNH or Succinate Controls		Bilirubin and DPNH or Succinate	
	O_2 Consumption/cu. mm.			
5 min.	40	40	1	1
10 min.	4	3	0	−1

* Data expressed in units of optical density.

This study opens up an approach to the definition of localization of bilirubin inhibition and suggests that the mechanism may be one of interference with electron transport.

95. Acute Erythroblastopenia Due to Chloramphenicol

ROBERT A. BRIDGES*; HUGH DILLON*; and ROBERT A. GOOD, Variety Club Heart Hospital and University of Minnesota.

It has been recognized that treatment with chloramphenicol may occassionally produce clinically significant blood dyscrasias. In a recent study based on serial bone marrow examination in patients being treated with chloramphenicol in high dosage (100 to 200 mg. per kilogram per day, we have observed the frequent occurrence of a striking disturbance in erythropoiesis. In most of the patients in whom the abnormality has occurred, the number of erythroblasts in the bone marrow has been strikingly reduced, while the other marrow elements have been unaffected. A variety of morphologic changes in the maturing red blood cells have been observed during the development of this phenomenon and these will be described. It appears that a period of treatment lasting two to three weeks is necessary before this anomaly develops to an almost complete suppression of erythropoiesis. Erythroblast counts in the marrow in those affected are often reduced to less than 1% of the nucleated cells (normal 10% to 20%). That the abnormality observed is a pure toxic phenomenon, and not due to an allergic mechanism as has been postulated for other hematopoietic disorders that occur in rare instances during chloramphenicol therapy, is evidenced by the prompt and complete reversal of the phenomenon following the cessation of treatment. With cessation of treatment, there occurs an intense normoblastic proliferation in the bone marrow and reticulocytosis, plus a leukoerythroblastic reaction in the peripheral blood. These changes are followed by return of erythropoiesis to normal.

We and many others have for several years used chloramphenicol in large doses to treat severe infections, especially those due to Hemophilus influenza. With only casual observation, we have not previously been aware of this effect on hematopoiesis. It appears that suppression of erythropoiesis by treatment with chloramphenicol, although not uncommon, becomes of clinical significance only when extended over a prolonged period. However, these observations provide a warning that large dose chloramphenicol therapy is frequently toxic to erythropoiesis and should be used with caution. The metabolic derangements reflected by the morphologic observations reported herein are currently under investigation in our laboratories. Studies concern metabolism of porphyrins, iron, and erythropoietin.

96. Limitations of the Postulate of Block of Hydrogen Ion Secretion in Explaining the Renal Action of Acetazoleamide

W. A. BRODSKY and R. SATRAN,* Louisville.

A reproducible pattern of increment of bicarbonate excretion versus dose of acetazoleamide was found in anesthetized dogs under conditions of normal pH and CO_2 content of serum and under conditions of metabolic alkalosis. However, the action of acetazoleamide was reduced to 1/2 to 1/6 of that expected when given to dogs under con-

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ditions of both metabolic and respiratory acidosis. Thus, diamox injection in a dose of 100 mg. per kilogram of body weight induced maximal increments of bicarbonate excretion of 400 to 500 micromoles per minute in normal dogs, and of 100 to 150 micromoles per minute in acidotic dogs. The suppression of drug action during acidosis was independent of glomerular clearance and of serum levels of bicarbonate and sodium. A scheme of diamox-induced block of hydrogen ion secretion must be fortified with certain forced assumptions to account for the data, e. g., 1. Even uncatalyzed reaction rates of CO_2 might be rapid enough to feed hydrogen ions into the "pump" at an unspecified but low intracellular pH during serum acidosis, and *2.* Perhaps only a small fraction of the hydrogen ion process is blocked during acidosis. However, recent evidence has cast doubt on the usually postulated role of carbonic anhydrase in the process of urine acidification: 1. Dogfish kidneys, devoid of carbonic anhydrase, produce acidic urine; *2.* Chlorothiazide, a potent in vitro inhibitor of C. A., and thiocyanate, a weak in vitro inhibitor, had no effect on renal acidification; 3. The theoretical maximal value of H^+ ions produced by cell metabolism is derivable from the overall oxidation of glucose,

$C_6H_{12}O_6 + 18\ H_2O + 6\ O_2 \rightarrow 6\ CO_2 + 24\ H^+ + 24\ OH^-$,

where the immediate source of such H^+ is not CO_2, but some unspecified metabolite of the Krebs cycle.

We propose, herewith, an alternate hypothesis of drug action—that of "bicarbonate block"—the elements of which are: 1. Diamox blocks a system for reabsorption of HCO_3^- ions as such; *2.* This ionic transport is not dependent on carbonic anhydrase. Such a scheme enables one to account for the suppression of diamox action during acidosis with but one additional requirement, i. e., that reduction of serum pH stimulates an increase in the rate of reabsorption of HCO_3^- ions. This requirement has considerable experimental foundation even though the exact mechanism of bicarbonate transport has never been defined.

97. Magnesium, Sodium, and Potassium in the Serum of Mothers and Their Newborns

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Albany Medical College
Introduced by PAUL R. PATTERSON.

Very little data on serum magnesium levels in pregnant women or in infants have appeared in the literature, and none could be found comparing levels of this cation in mothers at term and their newborn infants. It is known that approximately 20% of serum magnesium is protein bound, and since well-marked mother-newborn protein fraction differences exist, the authors were interested to note whether a transplacental magnesium gradient might also exist.

As various workers have shown slightly higher or much higher potassium levels in newborns than in adults, the present authors have determined to what extent, if any, the elevated potassium levels are related to maternal levels at birth. Similarly, newborn sodium levels were compared to the mother's level at term rather than to normal adult values.

These cation determinations were made on *20* mother-newborn pairs at the time of delivery.

No transplacental gradient was found for magnesium or sodium. However, magnesium values for mothers at term were significantly lower than values for this ion in *20* nonpregnant females of the same ages. This significant hypomagnesemia of pregnancy may be related to the hypervolemia of pregnancy or may be associated with the protein fraction changes in pregnancy.

Serum potassium was significantly higher in cord blood in all *20* infant-mother pairs, although cord potassium levels were not as high in this series as in others reported in the literature. Since potassium is a product of cellular catabolism and in fetal life can be excreted only by the maternal kidney, the higher cord levels may be thus explained. It is also possible that the relative anoxia of the term fetus could contribute to the elevated newborn potassium levels.

98. Observations on Some Serum Components In Mothers and Their Newborn Infants

DAVID F. BROWN*; EDWARD GILLIE*; ROBERT B. MCGANDY,* and JOSEPH T. DOYLE,*
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Introduced by PAUL R. PATTERSON.

Serum was obtained from 50 infants at birth (cord blood) and from their mothers at term. Levels of total protein, α-1, α-*2*, β- and γ-globulins, α- and β-lipoproteins, total lipids, and total cholesterols were measured. In infants total proteins were lower, albumin levels were higher, and γ-globulin levels tended to be equal in comparison with maternal levels. It followed that the lowered total globulin levels in infants must be due to lowered levels of one or more of the remaining globulin fractions. Analysis revealed that α-1, α-*2*, and β-globulins in infants were all lower. Total lipids and α and β lipoproteins were lower in the infants. However, α lipoprotein formed a greater percentage of total lipoprotein in the infants than in the mothers. Total lipid and lipoprotein determinations carried out on *20* of the same infants four days after birth revealed absolute increases over birth levels. Percentage α lipoprotein was lower at four days than at birth. Infant cholesterol

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levels at birth were approximately ⅛ maternal levels.

It is possible that the differences in levels that exist on both sides of the placenta reflect both molecular size and shape of the substances concerned. A large globulin molecule, for example, β lipoprotein (M. W. 1,300,000) may pass across the placental barrier less readily than the smaller albumin molecule (M. W. 66,500). The higher albumin levels in infants may indicate a compensatory mechanism to maintain oncotic pressure in the presence of low total globulins. As the majority of serum cholesterol is bound with β lipoprotein, it follows that one would expect the lower cholesterol levels that were found in infants.

Comparison of immunochemically determined γ-globulin levels in breast-fed four-day-old infants with levels in four-day-old artificially fed infants showed no significant difference. This suggests that colostrum makes no significant contribution to the infant's γ-globulin supply.

99. Immunological Study of Children Inoculated with Monovalent Asian Strain Influenza Vaccine

HENRY B. BRUYN and E. H. LENNETTE,* University of California

The latest antigenic variation in Influenza A, which has been termed Asian strain, has provided opportunity to study the immunological behavior of individuals in response to this strain in a monovalent vaccine. It has been shown that the childhood years are characterized by a narrow range of heterologous antibodies, reflecting scant experience with influenza, and that response to a specific antigenic combination will depend upon the strain experience of the individual child and the relationship of those strains with the new antigenic entity. The predominant response to a given antigen is of a booster type related to the strains with which the individual has had previous experience.

A group of 133 children were inoculated with monovalent Asian strain influenza vaccine by various routes and dosage plans. Strain-specific hemagglutination inhibition antibody titers against swine, PR8-1931, FM1-1947, and Asian-1957 strains of Influenza Type A were determined before and after inoculation. The heterologous and homologous responses were measured. A group of 70 children who were similarly evaluated in 1947 using a bivalent vaccine containing PR8-1931 and Lee B strains is contrasted and compared with the present group.

The absence of FM1-1947 strain antibodies in the group of children studied in 1947 and of the 1957 Asian strain antibodies in the group studied in 1957 is of interest. The immunological response to 1957 Asian strain depends upon repetition of inoculation while the response to a strain closely related to those with which the child had had past experience was adequate following a single inoculation in the 1947 group.

The complement fixation antibody titers were also studied, since they reflected broad antigenic relationship among various members of the Type A group and the response to a new antigenic variant of Type A Influenza such as the Asian strain was apparent.

The intradermal route of inoculation was found most adequate when two inoculations were used and was entirely comparable to the subcutaneous route.

It is concluded that the response of children and probably of young adults to inoculation with a new strain of Influenza A will depend upon repetition of the inoculation, especially when the new strain contains few of the antigenic components found in strains of more recent occurrence. It is further suggested that the total antigenic content of a single inoculation is less important than the repetition of inoculation.

100. BCG Vaccination in Newborns

ETHEL CERMAK,* Albany Medical Center
Introduced by PAUL R. PATTERSON.

In 1948, following the discovery in Albany Hospital of a high mortality rate from tuberculosis in noncontacts under 5 years of age, we undertook to vaccinate with BCG all newborns whose parents agreed to cooperate in a five year follow-up.

During the next two and one-half years, of 3890 live births, 1778 (46%) received BCG vaccine by resident pediatricians trained in the Birkhaug multiple puncture method. Thirteen (0.7%) failed to take, and 460 (26%) never returned for any follow-up. Of 1315 takes, 103 (7.7%) failed to convert the tuberculin reaction to positive, 15 in families with a history of tuberculosis, 2 of which were active. Thirty-nine took but did not return for further follow-up. Eleven-hundred-seventy-three (89%) converted and were followed for a variable time; 600 (51% or 34% of original group) for five years; 473 (40% or 27% of original group) for one to four years. Of these 1173, 432 (37%) converted and returned to negative by five years; 237 (20%) remained moderately reactive for five years; 471 (40%) remained reactive as long as they were followed, and 33 (2.8%) increased their reactivity during the five year study. Of these latter, three developed roentgenographic evidence of pulmonary tuberculosis which cleared. There were no untoward local reactions in any of the cases followed.

In the study group there were 15 deaths (0.84%), 53% confirmed by autopsy. Of the 2112 cohorts there were 19 deaths (0.90%), 47% confirmed by

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autopsy. Tuberculosis was not found in either group.

Graphs depicting the incidence of tuberculosis over the past 20 years have been prepared. From 1937 through 1947 there were 47 cases of tuberculosis in children under five years of age admitted to Albany Hospital of which 13 (28%) died. From 1948 through 1957 there were 65 cases with 14 deaths and 2 left decerebrate (25%). Thirty (46%) of these had no known contact with an open case. The pattern of tuberculosis in this community shows a continued high death rate in the first five years of life which defends the role of BCG vaccination in an overall tuberculosis control program.

It is clearly feasible to include BCG vaccination in the newborn nursery as one of the routine preventive immunizations.

101. Origin of Blood Ammonia in the Newborn

R. L. CLEMMENS * and S. P. BESSMAN, Maryland

A study of blood ammonia in newborn infants and prematures shows levels equal to or higher than those found in normal adults. The association of high blood ammonia levels with kernicterus makes it necessary to find the sources of blood ammonia in the newborn for proper therapy.

Normal sources in the adult are the gut, muscle contraction, and renal activity, related to urinary ammonia formation. Studies of gastric ammonia and urease activity in newborns with and without erythroblastosis fetalis reveal no significant formation of ammonia in the stomach. Studies of meconium also show little ammonia content or formation. Data will be presented which suggest by elimination the formation of ammonia by the renal tissues as the major source of blood ammonia in infants both with and without erythroblastosis. The significance of this in relation to the urinary excretion of ammonia will be discussed.

102. Urinary Steroid Excretion Patterns in the Neonatal Period

ELEANOR COLLE * and ROBERT A. ULSTROM, Minnesota

Twenty-four hour urine specimens have been collected from (1) well infants during the first month of life, and (2) infants having major surgery during the first month of life. Three fractions have been analyzed: the methylene chloride extract of the untreated urine, the methylene chloride extract after incubation with β-glucuronidase at pH 4.7, and the methylene chloride extract of the urine after acidification at pH 1.5. The methylene chloride extracts have been aliquoted to permit estimation of quantitative patterns by means of paper chromatographic techniques. In addition, that percentage of the free fraction Porter-Silber chromagens contributed by hydrocortisone has been estimated using an isotope-dilution technique.

The excretion of Porter-Silber chromagens (hydrocortisone equivalents) by the well infants varied over a wide range (.050 to .600 mg/24 hr.). Most of the values were, however, low when compared to adult values if comparison is made on the basis of surface area. In general, the ratio of free:glucuronide conjugated Porter-Silber chromagens was high in the infant, ranging from .05 to 6.0.

The steroid excretion in the surgical group was also variable. The youngest (less than one week of age) infants had excretion values well within the range of that found for the well infant. No sharp increase on the day of surgery was found. One infant operated upon during the second week of life had values three and one half times that found for any of the well infants. In addition, this child showed the highest value on the first surgical day with declining values in the subsequent period. ACTH stimulation in this infant one week after surgery resulted in excretion values no higher than those found on the first surgical day. The ratios of free:glucuronide-conjugated steroids in this infant were low.

Qualitative studies of these urines from the surgical group indicate that hydrocortisone, cortisone, and tetrahydrocortisone contribute to the Porter-Silber chromagens in the free fraction; tetrahydrocortisone, tetrahydrohydrocortisone and dihydrohydrocortisone to the glucuronide-conjugated Porter-Silver chromagens. Of considerable interest is the finding of further release of tetrahydrocortisone, tetrahydrohydrocortisone and dihydrohydrocortisone by acidic hydrolysis. In addition, the free fraction in both well and sick infants contains relatively large amounts of steroids more polar than tetrahydrohydrocortisone which have an active A ring (indicated by absorption of UV light, production of fluorescence after sodium hydroxide, and peak absorbance at 240 mμ in methanol).

103. Studies of Normal and Malignant Cells in Tissue Culture: Alterations in Primate and Nonprimate Cells Maintained in Tissue Culture as Measured by Antigenicity, Chromosome Number, and Tumor Production in Rats Pretreated with X-Ray and Cortisone

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The quantitative studies to be presented indicate that mammalian cells which undergo some unexplained change permitting continuous propagation in vitro acquire associated alterations not found in primary tissue culture cells of the same species.

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Complement-fixation tests show that eight continuously propagating tissue culture cell lines from man, monkey, and rabbit acquire antigens not present in fresh suspensions or first generation tissue culture cells from the same species.

Chromosome counts of primary tissue culture cells and continuously propagating cells of these three species show that the primary cells have the modal number characteristic for the species but that the "established" tissue culture cells have a modal number in the hypotetraploid range plus a wide spread of chromosome counts.

First generation tissue culture cells from normal tissues do not produce intraperitoneal tumors in rats pretreated with X-ray and cortisone. Ten "established" tissue culture cell lines maintained in continuous propagation in vitro produce solid intraperitoneal tumors in pretreated rats. The implications of these "alterations" will be discussed.

104. Glycogen Synthesis by Human Erythrocytes

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Sinai Hospital and Johns Hopkins

Although no glycogen has been found chemically in the mature human red cell, the present report indicates that there is some phosphorylase activity in red cells obtained from normal adults, cord blood, and one patient with the classical hepatic type of glycogen storage disease. This was determined by measuring the synthesis of glycogen from glucose-1-phosphate (G-1-P). As with muscle and liver phosphorylase, synthesis occurred only in the presence of both glycogen primer and G-1-P, and could be increased by the addition of adenosine-5-phosphoric acid, .001M (muscle adenylic acid). Heating, or substituting glucose, glucose-6-phosphate, or fructose 1-6 diphosphate for G-1-P abolished activity.

The synthesis of glycogen was followed by measuring the increase in intensity of iodine color. Since the intensity of the latter is dependent on the terminal chain lengths of the glycogen, the glycogen was also isolated and characterized by the total number of glucose units present. The phosphorylase activity has been of such variability that it has not been possible to date to use this system to demonstrate activation by glucagon or epinephrine, an original objective in the hope of using erythrocytes instead of hepatic tissue for studies of carbohydrate regulation in the newborn.

The phosphorylase activity of the erythrocytes from a patient with glycogen storage disease was within the range of normal. Although no glycogen had been found in the red cells of normal adults, newborns, a child with idiopathic spontaneous hypoglycemia, and an infant with reticuloendotheliosis, it was found in easily measurable amounts in the red cells of the patient with glycogen storage disease. This glycogen was isolated and characterized by determination of the iodine spectrum and intensity, and the total number of glucose units (anthrone). In all three respects, the glycogen closely resembled normal human liver glycogen (supplied by Dr. B. I. Brown). Since no glycogen is present in the trichloroacetic acid filtrates of normal red cells as determined by iodine color, the erythrocytes of patients with glycogen storage disease may be a source for isolating and characterizing the type of glycogen present in patients with different forms of the disease.

105. The Effect of Body Position on Respiratory Rate, Retraction Score, and Postnatal Weight Loss of Premature Infants

J. A. Cortner *; J. C. Dower,* and
W. A. Silverman, Columbia

A study designed to demonstrate any effect of position upon morbidity or mortality in the premature infant was made in the Sloane Nursery of Presbyterian Hospital from July 1957 through January 1958.

The group studied consisted of white and Puerto Rican infants with birth weights of 2000 to 2500 gm. and Negro infants of 1900 to 2500 gm. birth weights. During the first 36 hours of life, each patient admitted to the study was placed in one of four experimental positions (15 degree head-up prone, 15 degree head-up supine, 15 degree head-down prone, and 15 degree head-down supine) by random selection. Respiratory rate and retraction score were measured once in every six hour interval, and weight was recorded at 0-6, 18-24, and 30-36 hours of age. Patients received nothing by mouth during this period, and if indication for antibiotics arose, they were treated with a standard dose of penicillin and chloromycetin. A physical examination was performed by one of us at least once during the first 36 hours of life.

The four groups were comparable in the following ways: average birth weight; sex; race; serology, blood type, and Rh status of the mother; birth rank; mode of delivery, and obstetrical complications.

Some of the results are shown in the table below.

None of the differences of respiratory rate or weight loss between any two groups were statistically significant.

The retraction score proved of small value in a study of initially normal infants. Of all infants observed, 32.5% had measurable retraction on at least one occasion but only 7.1% showed what was arbitrarily considered retraction and, though

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Group	No.	Mean Respiratory Rate per Min.				Mean Weight Loss, Gm.		
		0-6 Hr.	12-18 Hr.	24-30 Hr.	0-36 Hr.	0-21 Hr.	21-33 Hr.	0-33 Hr.
Head-up	25	54.9	51.6	51.5	52.2	76.8	51.8	128.4
Head-down	26	53.4	53.8	55.9	54.7	74.6	45.6	120.2
Prone	25	57.1	52.7	53.5	53.6	61.6	55.2	116.6
Supine	26	51.1	52.2	54.0	53.0	80.4	42.1	131.5
Head-up prone	12	57.1	51.6	50.5				
Head-up supine	13	52.5	51.5	52.4	51.9			
Head-down prone	13	57.1	53.7	56.3	55.0			
Head-down supine	13	49.6	53.9	55.5				
1900-2250 gm.	19				56.1			114.2
2251-2500 gm.	32				51.6			130.0
Entire group	51	54.1	52.7	53.7	53.4	75.7	48.5	124.2

half of these were in the head-down prone group, this was not statistically significant.

These data are offered as evidence that the newborn premature (of birth weights between 1900-2500 gm.) during the first 36 hours of life seems to thrive equally whether supine or prone, or whether his crib is tilted head-up or head-down.

106. Isolation of a Cytopathogenic Agent Resembling CA Virus from an Infant with Croup

HENRY G. CRAMBLETT,* Iowa
Introduced by SAMUEL J. FOMON.

The association between infection with CA virus and the disease syndrome "croup" was reported in 1956 but has not been further substantiated.

In August 1957, a four-month-old infant was admitted to the University Hospitals of the State University of Iowa with croup. Bacteriologic cultures of the throat did not yield pathogenic organisms. From the throat washings of the patient, a cytopathogenic virus was isolated in monkey kidney-cell cultures. The cytopathogenic effect of the virus closely resembled that of the CA virus and was somewhat similar to the effect produced by mumps virus. The virus caused hemagglutination of newborn chick cells at 4 C, and reversal of the process occurred at 37 C. Reciprocal cross neutralization and hemagglutination-inhibition studies were performed with serums of the patient drawn during acute and convalescent phases of the illness, as well as with the virus isolated from the patient, the prototype CA virus and its immune antiserum. These tests suggest that the CA virus and the virus isolated from the patient studied in this laboratory are very similar if not identical. Studies of acute and convalescent serums of the patient failed to demonstrate increases in titer of antibodies against the adenoviruses, influenza Type A (Asian strain) virus, and mumps virus.

The combination of a sporadic case of croup occurring during the summer in a different geographic area and simultaneous infection with CA virus, as reported here, is important in further establishing the association between infection with CA virus (or group of viruses) and the clinical syndrome of croup.

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107. The Transfer of Estrogens and Carbohydrates Across Guinea Pig Placenta Using an in Vivo Perfusion Technique

JOSEPH DANCIS; MORTIMER LEVITZ,* and WILLIAM L. MONEY,* New York University and the Sloan-Kettering Institute.

Placental transport has been studied using an in vivo perfusion technique. A fetal guinea pig is delivered by hysterotomy and the umbilical cord is clamped. An umbilical artery and vein are cannulated and the fetal placenta perfused. The maternal circulation is left intact. There are many advantages to the technique among which are the elimination of fetal metabolism which often complicates transport experiments, and the opportunity for taking serial samples from the fetal circulation. The technique has been applied to the transfer of estrogens and of carbohydrates with the following results.

1. Estriol-C-14 is transferred rapidly in either direction across the placenta. The placental membrane appears to have a mechanism for releasing the steroid from the protein-binding in which it is transported in plasma.

2. There was no detectable transfer of estriol-C-14 glucuronide across the placenta in either direction under the conditions of the experiment. It would appear to be an advantage to the fetus to be unable to conjugate estrogens with glucuronic acid since this could lead to excessive retention in the fetal circulation. The situation probably reverses after birth because the kidney excretes the glucuronide more effectively than the free estrogen.

3. Glucuronic acid is transferred relatively slowly from the fetal to the maternal circulation as compared to glucose. This demonstrates the importance

of the carboxyl group in determining permeability and may be a major factor in the impermeability of glucuronides.

4. D-xylose, a pentose with the same spatial configuration as glucose but with one less carbon, is transferred from the fetal to maternal circulations three to four times more rapidly than L-xylose. This indicates a specific transport mechanism for carbohydrates. The carbons involved in this stereospecificity are under investigation.

108. Psychiatric Studies of Children with Epilepsy

CHESTER C. D'AUTREMONT*; ETHEL WALSH,* and GERALDINE RICKARD,* Harvard
Introduced by CHARLES JANEWAY.

The study is being conducted in the psychiatric clinic of the Children's Hospital, Boston. Our patients are referred from the hospital's Seizure Unit after a thorough work-up, for help with such problems as temper tantrums, school and learning difficulties, immaturity, inability to make friends, etc. They range in age from 6 to 17 years, and have at least one of the three major types of seizures. They are free enough of brain damage that it does not impair their capacity for new learning, abstraction, and judgment. This is, of course, a particular group of children, but we feel their problems may give clues to those of other children with seizures.

The diagnostic study consists of three interviews with the psychiatrist while the mothers are seen simultaneously by a social worker. The father is seen once, usually by the psychiatrist. The child is given tests of intelligence and personality. Children seen in treatment have been seen weekly.

In the cases seen, the mothers have shown strong fears to themselves or the child of death and separation, mobilized by the seizures but from our evidence, preceding them. Both the mothers and children see the seizure as a prototype of death. The threat of death and the fears and anxiety generate a wish to be rid of the child, a feeling so frightening that the mothers may avoid being with the child during the seizure. It is not surprising then, that the mothers show a strong need to control both the child and themselves.

The children seem threatened by their own aggression and that from the outside world, seeing the world as dangerous. The fears over aggression may be a factor in the severe learning difficulties which we found very commonly in the group. The children, in addition, see themselves as crazy and stupid. They fear the seizure may kill or injure them, or somehow lead to insanity.

The threat of separation is a great concern, and may often precipitate a seizure. On the other hand, we do not find "overprotection" in terms of overindulgence. There may even be a marked denial of the child's needs, in terms of illness or even safety.

With such fears and deeply rooted fantasies, both parents and children may have great anxiety about any procedures of diagnosis or treatment of their seizures. We feel that when realistic direct reassurance about some of the fears mentioned may be given. Careful explanation of such procedures as the EEG by the physician, and tolerance in its administration by the technician can be rewarding. Rather than give blanket reassurance, such statements, explanations and advice must be given with the individual child and family in mind.

109. The Pattern of Fecal Fat Excretion in Premature Infants

MURRAY DAVIDSON * and CHARLES H. BAUER,*
Cornell
Introduced by IRVING SCHULMAN.

Balance studies have indicated that in certain conditions manifested by steatorrhea the fecal fat is predominantly composed of saturated fatty acids. It has therefore been advocated by some that if the dietary fat is primarily made up of unsaturated fatty acids absorption will be improved. Other workers have suggested that the saturated fatty acids which are excreted in these disease states are not dietary but endogenous.

In order to ascertain the interrelations between the type and amount of dietary fat and other food constituents and their effect on the fecal fat in prematures, four groups of approximately 12 infants each were studied. One group was fed a half-skim-milk formula, a second an evaporated milk-dextri-maltose formula, a third an unsaturated fatty acid milk powder formula, and a fourth an evaporated milk-dextri-maltose formula fortified with protein powder so that the amount of ingested protein in the third and fourth groups was the same. In the two evaporated milk and the unsaturated fatty acid milk formulas the fat per 100 cc. was 3.2 gm. and in the half-skim-milk formula, 1.5 gm. All infants received approximately 150 cc. per kilogram per day of an 0.8 Calorie per cubic centimeter formula. Forty-eight hour stools were collected on all the diets.

There was a wide variation in the ability of these infants to absorb fat as measured by the coefficients of fat absorption. The values were all lower than those considered normal for full term infants in this laboratory. No significant difference could be observed between the percentage of fat absorption on any of the diets. The saturated fatty acids to total fat ratio remained remarkably constant irrespective of the amount of total fat excreted in the evaporated milk and skim milk diets. In the

* By Invitation

unsaturated fatty acid formula diet there was minimal saturated fatty acid excretion. On none of the diets was there "diarrhea" grossly or as measured by "wetness." There was no excessive nitrogen loss in any of the infants.

110. The Occurrence of Asian Influenza in a Group of Cleveland Families

FLOYD W. DENNY JR.; WILLIAM S. JORDAN JR.*; CONSTANCE CURTISS*; ROBERT OSEASOHN*; GEORGE F. BADGER,* and JOHN H. DINGLE,*
Western Reserve

The occurrence of an epidemic of influenza in the Far East and Southeast Asia due to a new and antigenically distinct strain of influenza A virus, and the probability that Asian virus would spread to the United States, prompted the reactivation of an illness study in a group of Cleveland families. The poulation of 60 families (308 persons) had been observed during the past 10 years. From Sept. 22 to Dec. 1, 1957, complete records were kept by the mothers of all illnesses in the families, and persons with respiratory illnesses were examined by a physician. At the time of each examination throat swabs were obtained, which were tested for the presence of influenza virus by inoculation of embryonated hens' eggs. Isolates were typed by the hemagglutination-inhibition test. Serum samples obtained from adults at the beginning of the study, and sera obtained from children in the spring of 1957, served as acute phase sera; all persons were bled at the end of the epidemic for convalescent phase sera. Influenza antibody titers were determined by both complement-fixation and hemagglutination-inhibition.

The epidemic began during the week of Sept. 29, reached a peak two weeks later, and was over eight weeks after the onset. Asian viruses were isolated from 52 of the 60 families (86.7%) and from 126 of the 308 members of these families (40.9%). These isolates were obtained from 43% of all respiratory illnesses sampled and from 92% of those thought clinically to be influenza. Isolation rates in different families ranged from 0% to 100%, ⅓ to ½ of persons being infected in the majority of the positive families. Rates for children were much higher than those for adults and were maximum in the 10 to 14 year olds; rates were lowest in fathers and highest in the school children. The secondary attack rate, as measured by virus isolation, was 34.9%. In persons from whom viruses were isolated, the symptoms and physical signs observed resembled those usually described for influenza and the clinical course was generally mild.

Serological studies showed that only one person, an adult, had had Asian influenza before the onset of the study. During the period of observation 60% of the entire population developed either complement-fixing or hemagglutination-inhibiting antibodies. Since ⅓ of the study group had received specific influenza immunization, and vaccinated individuals showed antibody responses, increases in antibody due to disease could be studied only in the unvaccinated group. Of this group, 53% showed significant increases of hemagglutination-inhibiting antibody and 46.8% increases of complementing antibody. The total serologic attack rate in unvaccinated persons was 54.7%. The incidence of infection by age or by family status as measured serologically followed the same pattern as noted for virus isolation, but at a higher level. As measured by serological tests, the secondary attack rate in 21 families in which no member had been vaccinated was 36.5%. In addition to the 126 persons from whom virus was isolated, 20 persons had serological evidence of influenza infection. Thus, 146 of the 308 adults and children in the 60 families were infected in less than three months, an attack rate of 47.4%.

The data presented here demonstrate the marked spread of a new infectious agent through a population in which there is no immunological protection, and show how this spread varies with age and family status.

111. Prolonged Pulsation of the Umbilical Cord After Birth a Presumptive Sign of Neonatal Distress

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Introduced by RUSSELL J. BLATTNER.

The umbilical cord of the neonate normally ceases to pulsate within 30 minutes after delivery. This report is concerned with the clinical course of 33 infants manifesting pulsations of the cord for longer periods of time (40 min. to 13 hr.). Pulsations are classified as "major" if visible or pounding in nature and "minor" if elicited only by palpation. They are synchonous with the heart rate, appear to be arterial, may be either continuously present after birth or cease entirely to reappear during distress. The umbilical vein may be distended or flat. Apparent venous pressure may or may not be elevated. In this series, a significant incidence of antepartum and intrapartum complication, fetal distress, and Caesarean delivery was present. Nineteen infants had difficulty in establishing respiration, and five expired. Only two infants had a completely uneventful neonatal course.

The patients have been classified into five groups according to the nature of the physical findings.

Group I (9 Cases) Transient or No Abnormality of Physical Findings.

* By Invitation.

Group	No. Cases	Complications of Pregnancy: Premature Labor	Complications of Pregnancy: Antepartim	Complications of Pregnancy: Intrapartim	Caesarean Section	Fetal Distress	Diff. Onset Resp.	Deaths
I	9	2	6	5	2	3	3	0
II	2	0	1	0	1	0	0	0
III	3	1	3	2	0	2	3	1
IV	3	0	2	1	0	2	3	2
V	16	7	10	10	8	9	10	3
Total	33	10	22	18	11	16	19	6
Percent	100	30.3	67	55	33	49	58	18

Group II (2 Cases) Hemorrhage from the Umbilical Cord after Ligation (three additional infants had cord bleeding associated with distress).

Group III (3 Cases) Extrinsic Factors Hampering Respiration (one infant had laryngeal obstruction, one pneumothorax, and one abdominal distention).

Group IV (3 Cases) Cardiac Failure on Delivery (poor quality of heart sound, coarse wet rales, liver enlargement, and response to digitalis). Two deaths—Rh erythroblastosis, bronchopneumonia.

Group V (16 Cases) Cardiopulmonary Distress Syndrome. Initial findings as follows: Dilation alae nasae, expiratory grunt, sternal retraction, regular labored respiration at a normal or slightly increased rate, loud clear monotonous regular heart sounds with an increased P^2 at usually a normal rate. With time this clinical picture changes variously. There were three deaths (2—hyaline membrane disease, 1—staphylcoccus aureus septicemia).

The common factor appears to be a disturbance of pulmonary blood flow. The continued presence of pulsation in the cord may indicate incomplete cardiopulmonary adjustment to air breathing. Routine inspection and palpation of the umbilical cord may be a procedure of value in the initial examination of the newborn infant.

112. Gonadal Dysgenesis in Infants and Children

ANGELO M. DIGEORGE* and JAMES B. AREY, Temple

Although gonadal dysgenesis (Turner's Syndrome, ovarian agenesis) after puberty is well recognized, its manifestations and pathogenesis have only recently become familiar to pediatricians. The Bonnevie-Ullrich Syndrome in infants is now firmly established to be frequently associated with gonadal dysgenesis. The development of the chromosomal sex typing technique of Barr now makes it possible to establish the diagnosis of gonadal dysgenesis in early infancy when a discordance of chromosomal sex and phenotypic sex is found.

The associated abnormalities of development which lead one to suspect the diagnosis have been frequently reported. The majority of these abnormalities are compatible with life and many may be considered as only minor defects. The present report describes the clinical and pathologic findings in six infants and children who died and in whom the diagnosis of gonadal dysgenesis was established by histologic examination of the gonads. The phenotype in all of the patients was female. The diagnosis of gonadal dysgenesis was suggested during life in three of the six patients on the basis of clinical manifestations and/or difference in the chromosomal sex and the apparent sex of the infants.

Variations in the histologic pattern of the affected gonads will be described. It is suggested that the frequency of gonadal dysgenesis is greater than heretofore suspected since severe and lethal congenital anomalies which may be associated with it result in early death of the patient. Additional evidence for this, based on other living patients with severe anomalies, will also be presented.

113. Hypoelectrolytemia in Cold Weather in Infants and Young Children with Cystic Fibrosis of the Pancreas

PAUL A. DISANT'AGNESE, Columbia

Five infants were admitted to Babies Hospital during winter months because of hypoelectrolytemia. Only one of them was previously known to have cystic fibrosis. Adrenal insufficiency was suspected in all and two were referred to this hospital after correction of the electrolyte abnormalities for study of this condition.

Two infants, four and eight months of age, respectively, were admitted as emergency cases because of dehydration not clearly explained by the amount of vomiting and diarrhea, although some had been present. Serum values for chloride, sodium, and potassium were reduced in both patients, in the first one to a marked degree. The diagnosis of cystic fibrosis was suspected in the first patient because of family history and later confirmed by laboratory tests. In the second infant the findings in the first case led eventually to the correct diag-

* By Invitation.

nosis. Appropriate studies showed satisfactory retention of chloride and sodium by the kidneys and normal values for 17-ketosteroid excretion.

Two additional infants, six and five weeks of age, respectively, were referred to Babies Hospital for study of their adrenal function because of previously corrected hypoelectrolytemia, not explained by vomiting or diarrhea. Because of the previous experience the diagnosis of cystic fibrosis was suspected and then confirmed by laboratory tests. Urinary excretion of 17-ketosteroids was normal.

In none of the four patients has there been recurrence of hypoelectrolytemia in a period of observation varying from four years to several months.

A fifth patient, 17 months of age, known to have cystic fibrosis was admitted twice to Babies Hospital within a few weeks, in both instances in a severe state of electrolyte depletion and dehydration. Both times moderate vomiting had preceded emergency hospitalization. Upon recovery conservation of sodium chloride by the kidneys was shown to be satisfactory and the urinary metabolites of adrenal hormones were within normal limits.

The possible mechanisms leading in a short time to profound salt and water depletion in infants with cystic fibrosis in cold weather are discussed. It is concluded that in the presence of hypoelectrolytemia in infancy, not satisfactorily explained, cystic fibrosis of the pancreas should be considered.

114. Cord Vein Blood Oxygen Saturations and Intelligence Quotients

DONAL DUNPHY*; VIVIAN PESSIN,* and LOREN MACKINNEY, Buffalo and Pittsburgh

Data derived from a study of 374 children, including 33 prematurely born, on the association of cord vein blood oxygen saturation at birth and psychometric test scores in the first five years of life will be presented. Cord vein blood was obtained within moments of delivery. The oxygen per cent saturation was calculated from the oxygen capacity and content determined by Van Slyke manometric gas analysis. The Cattell Infant Intelligence scale was used during the first years of life and the Revised Stanford-Binet psychometric test from two to five years.

Positive correlations between the oxygen saturation levels and IQ's were found. The correlation coefficients dropped from +.14 in the first year to +.09 in the fifth year; they were significantly different from zero in the first three years of life, but at ages four and five the differences were no longer significant. There is evidence that O_2 saturation levels at birth are directly related to subsequent intelligence scores, although the relationship is of low magnitude and diminishes with age. A positive relationship has not been demonstrated by other studies using smaller numbers of subjects.

As a possible measure of the oxygen economy of the newborn, the difference between cord vein O_2 saturation and arterial saturation was also investigated. Similar but smaller and not significant correlations with IQ's were found.

115. Heart Disease Associated with Hemosiderosis and Chronic Anemia

MARY ALLEN ENGLE; HENRY P. GOLDBERG*; GERTRUDE STERN*; IRVING SCHULMAN, and CARL SMITH, Cornell.

Transfusions may be life-saving for infants and children with severe refractory anemia, but during the second decade of life a complication of this form of therapy may cause death in heart failure. Ten such patients (nine with Mediterranean and one with aregenerative anemia) have developed cardiac enlargement, multiple auricular arrhythmias, often with auricular-ventricular block, and heart failure. Eight have died within a year of onset of cardiac symptoms. Two others are still alive a few months after the appearance of arrhythmias and congestive failure. Skin pigmentation, stunted growth, hepatosplenomegaly, and signs of massive iron overload preceded the cardiac symptoms. Postmortem examination demonstrated widespread iron deposition with the same distribution as in hemochromatosis. A comparable group of patients with severe anemia, treated in like manner, also show biochemical and biopsy evidence of hemosiderosis but have not yet developed arrhythmias or heart failure. The heart disease has proved refractory to the usual methods of treatment. Supportive therapy has consisted of digitalization, low salt diet, diuretics, and acidifying regimen.

116. Congenital Toxoplasmosis: Follow-Up Study

HARRY A. FELDMAN and LOUISE T. MILLER,* State University of New York, Syracuse

Information concerning the persistence of antibodies for toxoplasma can be obtained conveniently from cases of the congenital disease since the time of acquisition of the infection can be approximated. Follow-up questionnaires were sent to physicians who, in the past, had submitted sera and data from such cases to this laboratory. The questionnaires were designed to yield information concerning the degree and kind of handicap which survival from congenital toxoplasmosis had imposed on the child. In addition, new serum samples were requested from the mothers and children for titration by the dye and complement-fixation tests.

Historical information has been received from approximately 50 cases but their analysis is con-

* By Invitation

tinuing. Although two of the children are now in college, all have some handicap, either visual, intellectual, or motor, alone or in combination.

The serological behavior of both mothers and children are interesting in that dye antibodies persist at significant levels. Surprisingly, complement-fixing antibodies are detectable in most. These serological findings are consistent with a previous report from this laboratory that antibodies may be detected in significant levels in older children and young adults who have survived congenital toxoplasmosis.

117. Heat-Labile Antitoxoplasma Serum Factors in Fetal Circulations

HARRY A. FELDMAN and LOUISE T. MILLER,*
State University of New York, Syracuse

Sera obtained from adult animals and fowl (but not from humans or mice) can be shown to affect toxoplasma adversely in the dye test or by subsequent inoculation into mice. This reaction is independent of specific antibody and may be abolished by heating the serum for 30 minutes at 56 C, by sufficient dilution, or by treatment with zymosan. Fresh human serum does not have this effect except in the presence of specific antibody (activator).

Chick embryos were selected as experimental sources of the heat-labile toxoplasma effect. Embryos were bled by needle and syringe and the sera of four to six pooled for testing. Specimens were obtained from 9 through 14 day old embryos and then on the 16th and 19th days. Several chicks hatched and these were bled on the 1st, 2d, 6th, 9th and 12th days of life. None was found to have either the nonspecific or "activator" activities. Further studies are continuing to determine when the adult serum activity becomes measurable.

The sera of both a mother and her term baby were found to contain equivalent amounts of activator and complement. The same was true of a baby born after 37 weeks of gestation. However, the serum of an 18 week fetus contained no complement, a questionable amount of activator, and practically no toxoplasma antibody. In contrast, its mother's serum contained a normal amount of complement and at least eight times more toxoplasma antibody and activator. Other fetuses are being sought for similar study.

The absence of heat-labile serum factors from the fetal circulation at this stage of gestation and the paucity of antibodies from the maternal circulation may explain the increased "susceptibility" of the fetus to damage by toxoplasma. It may also play a role in those abnormal states, such as hemolytic reactions, which depend upon complement.

* By Invitation

118. The Postoperative Renal Excretion of Water in Infants

DANIEL C. FLEISHER*; WALLACE W. MCCRORY, and LEONARD BACHMAN,* Pennsylvania

Previous work has indicated a lack of agreement concerning the factors regulating the postoperative renal excretion of water in infants. The clinical importance of such knowledge is readily apparent.

To study this problem, water loads were administered to a group of infants ranging in age from 3 to 26 months immediately following minor surgical procedures. The patient's response to an identical water load (3% of body weight) administered at a time unrelated to surgery served as his control observation. A sustaining infusion was maintained during the study period to compensate for insensible losses of water. Serial determinations of serum sodium concentration and osmolality, and urine osmolality and volume were obtained. During the postoperative study urine collections were obtained in most patients by means of an indwelling urethral catheter. Free water clearances were calculated and employed as one index of the presence or absence of antidiuresis. The endogenous creatinine clearance was measured to estimate changes in the glomerular filtration rate.

Observations on five patients revealed the presence of a period of antidiuresis extending at the least four hours postoperatively. This was evidenced by the development of extracellular hypotonicity and the concurrent failure to excrete a dilute urine. No attempts to insure hydration prior to surgery were carried out in these subjects. The possible role of dehydration prior to surgery rendered the interpretation of these data difficult.

Five patients have also been studied who were well hydrated at the time they underwent surgery. One subject in this group responded in a normal manner (i. e., similar to his control response to a water load). The remanining four subjects exhibited periods of postoperative antidiuresis of varying duration. The occurrence of antidiuresis could not be attributed to prolonged postoperative depression of G. F. R.

The state of hydration is an important factor in determining the duration of the period of postoperative antidiuresis. It would appear from these observations, however, that it is not the only factor operative.

These studies are being continued with the aim of defining other causative factors of postoperative antidiuresis (type and length of surgical procedure and/or anesthetic, etc.).

119. Reproducibility of Three-Day Metabolic Balance Studies with Infants

SAMUEL J. FOMON; LORA N. THOMAS*; ROBERT L. JENSEN,* and CHARLES D. MAY, Iowa

Metabolic balance studies have been utilized for many years as an adjunct in evaluating diets for infants. Recently considerable skepticism has been voiced regarding the reliability of such determinations. In an attempt to determine the magnitude of the experimental error inherent in nitrogen balance studies of infants, we have performed two three-day metabolic balance studies consecutively while maintaining the intake of nitrogen nearly constant. By comparison of the data obtained in the two members of each pair of such consecutively performed balance studies it is possible to exclude the factor of variability between babies and to minimize the factors of variability in age and in intake of nitrogen. Results of the analysis of such data provide an approximation of the magnitude of the experimental error of the procedure.

Thirteen such pairs of metabolic balance studies have been performed with five infants ranging in age from 66 to 176 days. The retention of nitrogen of the infants ranged from as low as 300 mg. per day to as high as 928 mg. per day. The retention of nitrogen differed in the two members of a pair of metabolic balance periods by more than 100 mg. per day in three instances (159, 175 and 234 mg. per day, respectively) and the mean difference for the 13 pairs of periods was 105 mg. per day.

120. 11-β Hydroxylase Deficiency in Congenital Adrenal Hyperplasia in the Absence of Hypertension

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University of Rochester
Introduced by GERALD MILLER.

The demonstration of a deficiency in hydrocortisone synthesis and an excessive excretion of pregnane-3,17,20 triol in the urine of patients with congenital adrenal hyperplasia has suggested that the underlying defect in adrenal function is a deficiency in 21-hydroxylation. More recently a patient with a hypertensive form of congenital adrenal hyperplasia has been described by Eberlein and Bongiovanni. Their patient had an apparent block in 11-β hydroxylation. Large quantities of tetrahydro-S, and lesser amounts of pregnane-3-α, 17-α, 20-α, 20-tetrol and tetrahydrodesoxycorticosterone were found in his urine. The hypertension was attributed to adrenal secretion of desoxycorticosterone.

The study reported here concerns a patient with congenital adrenal hyperplasia without evidence of hypertension who excreted 15 to 21.5 mg. of blue tetrazolium-reducing C-21 steroids. An aliquot of the neutral ether extract was fractionated by paper chromatography. No traces of normal 11-oxygenated C-21 compounds were detected. Tetrahydro-S accounted for 68% of the high total corticoid value. Other α-ketolic steroids were isolated and studied by (1) comparison of their chromatographic behavior in the free state and as an acetate with that of authentic adrenal cortical steroids and their acetates respectively, (2) chromophoric reactions to spot testing reagent, (3) lack of ultraviolet absorption in absolute methanol, and (4) absorption spectra in concentrated sulfuric acid. These three compounds were most likely dihydro-S, tetrahydrodesoxycorticosterone, and dihydrodesoxycorticosterone. The urinary steroid pattern is indicative of a deficiency of 11-β hydroxylase in a patient with a congenital form of adrenal hyperplasia without hypertension. Both the normotensive and the hypertensive patient with congenital adrenal hyperplasia excreted similar amounts of desoxycorticosterone metabolites. Therefore it is unlikely that the differences in blood pressure can be explained by differences in the amount of desoxycorticosterone produced. An individual with congenital adrenal hyperplasia due to deficient 11-hydroxylase may or may not have hypertension. The accessory factors which determine the difference in blood pressure are not known.

121. Studies in Cystic Fibrosis of the Pancreas: Amino Acids in Sweat

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Introduced by HARRY SHWACHMAN.

Thermally induced body sweat was subjected to amino acid analysis using paper chromatographic techniques. The two dimensional system was employed with phenol-water and collidine-lutidine as solvents. Circular filter papers were also used with ethanol-acetic acid and butanol-pyridine.

Over 75 sweat samples were analyzed, 25 were from patients with cystic fibrosis and the remainder were from patients with a great variety of other conditions as well as healthy persons. Seventeen amino acids have been found in sweat. A synthetic sweat containing approximately proportional amounts of these amino acids have given identical chromatographic patterns. The amino acids in sweat from cystic fibrosis patients stain more intensely than the control group, suggesting a quantitative distinction which is being investigated. In addition, there is some trailing in slower migrating amino acids which we suspect is due to the high concentration of salt present in the sweat of the cystic fibrosis patients. This distortion of aspartic acid, glutamic acid, serine, and glycine

* By Invitation.

	% of Total	Number of Bacteria/Ml. of Urine				
		.0	.10^{1-3}	.10^{3-4}	.10^{4-5}	.10^{5-6}	More than 10^6
Normal Females	63						
Up to 1 year	31.4	1.8%	18.5%	5.5%	5.5%	0%	1.8%
From 1 to 6 yrs	18.5	7.4%	3.7%	7.4%	0%	0%	0%
From 6 to 14 yrs	13	3.7%	5.5%	1.8%	0%	0%	0%
Normal Males	37						
Up to 1 year	9.2	0%	3.7%	1.8%	0%	0%	1.8%
From 1 to 6 yrs	11.1	1.8%	3.7%	1.8%	1.8%	1.8%	0%
From 6 to 14 yrs	16.6	11.1%	5.5%	1.8%	0%	0%	0%

Normals recatheterized: 4 50% remained under 10^3 colonies/Ml.
25% remained over 10^6 colonies/Ml.
25% went from 10^3 to more than 10^5 colonies/Ml.

plus other features such as the constant presence of histidine makes the chromatographic pattern of cystic fibrosis distinct.

122. Quantitative Urine Bacteriology in Children

G. D. Godenne*; C. B. Morrison,* and R. E. Cooke, Johns Hopkins

Kass has demonstrated quantitatively the bacterial content of urine in asymptomatic adults. Approximately 87% of the adults, considered to be free of urinary tract infection, had counts less than 10,000 colonies per milliliter.

Application of a similar technique to urines of approximately a hundred children, who were free of symptoms of urinary tract disease, yielded comparable results. Ages ranged from a few days to 14 years. The patients were primarily less than 8 years of age. Sixty three per cent of the children were females, and were catheterized. Among the 37% of the group, which represents the males, ⅓ were catheterized, and midstream specimens were obtained on ⅔.

A 1/10 and 1/20 dilution of urine in nutrient agar was carried out within 15 minutes of collection. Colony counts were performed after 24, 48, and 72 hours of incubation. Urines were also examined microscopically for white blood cells.

The results are given in Table 1.

There was a discrepancy between the presence of pyuria and bacterial counts over 10^6 per milliliter.

It is concluded that quantitative bacteriologic analysis of urine is of value in children, as well as adults, in differentiating bacterial contamination during collection from infection of the urinary tract.

123. Placebo Versus Reserpine: Comparison of Value in Controlled Study of Maladjusted High-Grade Retarded Children

Bruce D. Graham; Sidney Rosenblum*; Roger Callahan,* and Richard Deatrick,* Michigan

It was the purpose of this study to determine if a tranquilizing drug (Reserpine) would be effective in a group of high-grade retarded children manifesting various behaviors that are generally reflective of personal and social maladjustment.

From a population of 800 high-grade retarded children in a residential treatment training center, 30 children were chosen who seemed to present the most severe problems in the joint opinion of the School Principal, Director of the Psychology Department, and Child Care Director. Their intellectual level ranged between 50 and 85. Matched on the basis of age, sex, IQ, and length of residence, the children were divided into three groups randomly assigned for treatment procedure. Group I received Reserpine for four months, Group II, Placebo for four months, and Group III, Reserpine for two months and Placebo for two months.

The following measurements were made during the observations.

1. Revised Stanford-Binet Test for Intelligence.
2. Measure of Manifest Anxiety.
   a. Children's manifest anxiety scale
   b. Children's anxiety pictures
3. Behavior Rating Scales.
   a. Gardner-Thompson Rating Scale
   b. Graphic Rating Scale in the school and cottage.

Inspection of results obtained reveals that these children receiving tranquilizing medication showed no greater improvement than did those receiving Placebos. The significance of any exceptions will be discussed as will the Placebo effect.

124. Studies on Glucuronide Conjugation and Diurnal Variation of Urinary 17-Hydroxycorticosteroids in the Neonatal Period

Robert Greenberg*; Lytt I. Gardner, and Artelissa A. Lippert,* State University of New York, College of Medicine, Syracuse

The metabolism of hydrocortisone involves a sequential process of reduction in ring A, followed by conjugation with glucuronic acid. Initially there are two stepwise double-bond saturations in ring A in which TPNH functions as the hydrogen

* By Invitation

donor. This is catalyzed by highly specific enzymes. Following this there is a third reduction, easily reversible, of a 3-keto group to a 3-α-alcohol. This reduction is catalyzed by a less specific enzyme, with either DPNH or TPNH acting as coenzyme. Once tetrahydrocortisone has been formed, there takes place conjugation of this steroid with glucuronic acid. This latter reaction lacks the degree of specificity inherent in the ring A reductions, and is known to occur with many organic compounds.

Using bilirubin as the substrate, other workers have recently found that microsomes from the fetal rat or guinea pig can scarcely form bilirubin glucuronide, whereas microsomes from the adult animal experience no such difficulty. Furthermore, premature and full-term human infants have been found by A. Brown et al. to excrete very small amounts of urinary glucuronides during the first days of life, with a gradual increase by the end of the first week.

In our studies the determination of free and conjugated 17-hydroxycorticosteroids was carried out on the urine of newborn premature and full-term infants within the first three days following birth. The method of Glenn and Nelson was used, and analyses were made before and after the addition of β-glucuronidase. The data indicate that over 90% of the newborn's urinary 17-hydroxycorticosteroids are excreted in the conjugated form. The results suggest that the newborn possesses a functional mechanism for the conjugation of corticosteroids as glucuronides and/or that the large percentage of conjugated steroids in relation to free in the newborn's urine is a reflection of the placental transmission of maternal conjugates. It is possible in the competition of corticosteroids versus bilirubin for glucuronide conjugation that the corticosteroids may enjoy relatively preferential conjugation.

In the present study data were also obtained concerning the diurnal variation (if any) of urinary corticosteroids in the newborn period. A diurnal variation of plasma 17-hydroxycorticosteroids in adults has been clearly shown in studies of others. Maximum plasma values are observed between 5 A. M. and 8 A. M., with the peak of urinary 17-hydroxycorticosteroid excretion occurring about two hours later. There is good evidence that this pattern of diurnal variation takes place as a result of changes in the rate of production of adrenocortical steroids.

In our study all urine passed by normal full-term newborns was collected in eight hour partitions during the first three days of life (8 A. M. to 4 P. M.; 4 P. M. to midnight; midnight to 8 A. M.). Free and conjugated urinary 17-hydroxycorticosteroids were determined as previously described. No consistent pattern of diurnal variation of urinary 17-hydroxycorticosteroids was found. It is suggested that this lack of diurnal variation represents a very early stage in the maturation of hypothalamic-pituitary function.

125. Effects of Thyroid Hormone on the Maturation of the Central Nervous System

MAX HAMBURGH,*
Albert Einstein College of Medicine
Introduced by EDNA H. SOBEL.

The observation in children with cretinism that replacement therapy with thyroid hormone may be more effective in raising cerebral function to normal levels if started very shortly after birth, suggests that in the absence of thyroid hormone irreversible changes are taking place in nervous tissue during its period of maturation. The experiments to be reported here were undertaken to answer specifically the following questions: 1. Are all parts of the central nervous system sensitive to the presence or absence of the hormone during its maturation? 2. Is the maturation factor of the hormone required continuously during the period of development of the tissue, or is its action and influence limited only to a very specific and critical phase in the sequence of differentiation? 3. What is the relationship between hormonal environment and genetic constitution with respect to the differentiation of the nervous system?

Mice of the DBA/2 strain were chosen because they are excellent breeders and exhibit a high incidence of a common neuropathological syndrome "audiogenic seizures." The experiments were subsequently extended to strains C57 B1/10, and A Jax.

Mice were injected with 2μg. of tri-iodothyronine every second day. Injection was started in one series from birth and in another from the 7th day of age. In a third series of experiments animals were thyroidectomized at birth by injection of 100μc. of I^{131}.

The age at which the corneal reflex, the startle response, and the swimming reflex emerge, as well as the onset of the strain-characteristic audiogenic seizures and the ability to develop a conditioned response were recorded as indicators of completed neural maturation of certain sensory and reflex patterns.

Our results indicate the following.

1. Thyroid treatment accelerates, while thyroidectomy delays the mean age at which the corneal and startle response normally appear. Thyroid treated animals of the DBA/2 strain responded with strain characteristic audiogenic seizures 3 to 4 days earlier than their control littermates. Thyroidectomy, on the other hand, did not affect the onset of seizures

*By invitation

in susceptible animals. The proportion of animals affected within the strain is determined by heredity and remained uninfluenced by thyroid treatment.

2. There is no clear evidence of the effect of excess of thyroid hormone or its lack on the emergence of the swimming reflex and the ability to perform a simple conditioned response.

3. Thyroid treatment initiated on the 7th day of postnatal age instead of at birth was equally effective in accelerating those types of behaviour which yielded positively to hormone treatment at an earlier age. Conversely, thyroidectomy did not suppress the emergence of any behaviour pattern, but merely delayed its rate of maturation.

From our results it can be tentatively concluded that the thyroid hormone affects different components of the central nervous system to a different degree and that some structures may be altogether insensitive to the presence or absence of the hormone. Hormone treatment can affect differentiation of tissues only within the limits set by the genetic factors operating.

The further observation that these "acceleration" effects could be elicited if hormone injection was delayed for as long as seven days after birth suggests to us that during development the hormone acts as a "trigger" activating various components of the central nervous system.

126. The Response of Nonanemic Children to the Parenteral Administration of Iron

DENMAN HAMMOND*; CHARLES MCKEAN,* and WYLDA HAMMOND,* with the Assistance of PHEBE MILLER and ARLENE MURPHY,
University of California
Introduced by HOWARD A. JOOS.

Twenty children with normal values for hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration were given 200 mg. of iron by intramuscular injection. Response was assessed by determining hemoglobin, hematocrit, MCHC, serum iron, total iron-binding capacity, % saturation of iron-binding protein, serum copper, and free erythrocyte protoporphyrin prior to injection, six weeks following injection and again six months later. Bone marrow specimens suitable for the assessment of stainable iron and the enumeration of sideroblasts were prepared before injection and again six weeks after injection.

Evaluation of the baseline data revealed values for hemoglobin, hematocrit, and MCHC of this selected group distinctly above reported mean values for a normal population over the age range studied. None of the patients could be suspected of an iron deficiency of the hemoglobin compartment.

The mean values for iron-binding capacity, serum iron, saturation of iron-binding protein, and free erythrocyte protoporphyrin were considered normal. However, individual values of some patients exhibited a pattern usually considered indicative of depletion of iron stores. The mean value for serum copper was elevated.

Only four patients had demonstrable amounts of stainable iron in pretreatment marrow preparations; however, sideroblast counts on the same specimens revealed stainable iron granules in 4% to 75% of the late nucleated erythrocytes.

Six weeks after injection, the mean cytologic values were not significantly altered. Several biochemical alterations appeared to be significant. Mean values for iron-binding capacity, serum copper, and erythrocyte protoporphyrin were decreased, whereas saturation of iron-binding protein was increased. Serum iron was not significantly altered. Stainable iron in the post-treatment marrow specimens was decidedly increased in every instance. Percentage of sideroblasts was decidedly increased in all but two post-treatment specimens.

Six months after injection there was still no significant alteration in mean cytologic values. Mean values for all biochemical determinations reverted toward pretreatment levels with the exception of erythrocyte protoporphyrin which remained essentially unchanged from the six week value.

Several patients exhibited significant deviations in individual response which could be related to changes in their clinical course.

The data indicate that in the absence of anemia, the state of iron stores as detectable by currently available methods may vary from none to considerable quantities. Biochemical determinations which reflect a normal state of iron nutrition may be further influenced by the parenteral administration of iron; however, with time the extent of these alterations decreases.

The significance and interrelationship of these various methods for assessment of the state of body iron stores will be illustrated.

127. Total Body Fat in Adolescent Boys: A Preliminary Report

FELIX P. HEALD*; ROBERT SCHWARTZ; EDWARD E. HUNT JR.,* and PER HELLIESEN, Harvard

This project was designed to explore all available methods for measuring total body fat in adolescent boys, in the hope that the results would further an understanding of total body fat changes during adolescence.

Twenty-four boys were selected by a layman from a boys' club. Four boys, who did not look obese and who could swim adequately, were selected at each age between 12 and 18. All measurements of total body fat were performed on the same day.

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The following methods of measurement were used in estimating total body fat.

1. Total Body Water: This was determined by the heavy water (HDO) method. Two and three hour samples were obtained and analyzed by the mass spectrometer.

2. Densitometry: Specific gravity was determined by dry weight and by underwater weight. Residual lung volume, which is crucial for accurate densitometry, was determined both out of water and under water. Technically this proved to be the most difficult measurement.

3. Skin Fold Thickness: Multiple skin fold sites were measured by constant pressure calipers. Certain sites were selected which would correspond to the roentgen method of measurement of fat in the arm and chest. Multiple skin fold measurements determine relative amounts of subcutaneous fat, not total body fat, and its individual distribution.

4. Roentgen Measurements: A standardized X-ray of the arm was taken with emphasis on soft tissue contrast. In addition an A-P X-ray of the chest was done so that the subcutaneous fat in the lateral chest wall could be measured. Hand-wrist X-rays were taken to determine skeletal age.

5. Somatotype: A somatotype photograph was taken of each boy so that a permanent record of his physique would be available.

In evaluating the results of this study, it is felt that in biological data of this sort one should use skeletal age rather than chronological age as an index of biological maturity.

As skeletal age increased through the range from 11.5 to 18.0 years, both total body water (its absolute value) and its expression as percentage of total body weight, increased. Conversely, as skeletal maturation increased, total body fat, as calculated from total body water, decreased.

Total body fat, as determined by densitometry, was less in those of greater skeletal age. There was, therefore, a correlation in body fat changes as determined both by total body water and by densitometry. But at present there is no absolutely accurate way of determining total body fat short of direct tissue analysis, so that the significant difference in absolute values for total body fat, as estimated by densitometry or by total body water, could not be resolved.

Neither single skin folds, nor a multiple skin fold index, parallel the changes in total body fat described above. However, they serve to differentiate the relative thickness of subcutaneous fat between boys in this study.

A simple but accurate clinical technique for estimating total body fat is under consideration.

128. Tridione Nephrosis in Rats

WALTER HEYMANN * and DONALD B. HACKEL,*
Western Reserve
Introduced by WILLIAM M. WALLACE.

The nephrotic syndrome may be noted in children treated with Tridione. The experimental production of a similar renal disorder has, however, thus far not been successful in animal experiments. In our studies 21 rats have survived daily oral administration of Tridione long enough to serve as experimental material. They were given 37 to 47 mg. of Tridione daily by tube. Related to body weight this corresponds approximately to 6 to 8 times the maximal dose recommended for older children. Six to eight months after Tridione administration was started, nine animals developed a mild to moderately severe nephrotic syndrome with proteinuria varying between 120 and 550 mg. per 24 hr. Mild hypoproteinemia and hyperlipemia were present, while azotemia, creatinine retention, or hematuria were not noted. Histological changes in the kidneys will be described, which support the diagnosis of a nephrotic lesion. In contradistinction to the disease in man, the renal disease in rats does not seem to subside after Tridione administration has been discontinued. The effect of steroid administration is being studied at present and will be reported.

129. The Relationship of γ-Globulin Levels to Antibody Response in the Infant

RUTH HEYN * and CATHERINE J. CARROLL,*
Michigan
Introduced by BRUCE D. GRAHAM.

The problem of whether or not the "physiological" drop in the level of γ-globulin during the first six months of life has any relationship to the antibody response in the infant prompted the study of the levels of γ-globulin and poliomyelitis antibodies in this age range. Further interest was initiated by the variable antibody response of the young infant to the administration of poliomyelitis vaccine during this period.

Serum γ-globulin levels were done on a series of mothers and their infants in correlation with a poliomyelitis vaccine study. The globulin levels were determined by a modification of the ammonium sulfate salting-out procedure described by Jager and Nickerson.

The mothers were divided into two groups. One group received two injections of poliomyelitis vaccine during the third trimester of pregnancy. The other two received no vaccine and acted as a control group. Antibody levels and γ-globulin levels were obtained on the mothers prior to receiving the vaccine and again at the time of delivery. The infants in each of these groups had the same de-

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terminations performed on serum from cord blood the first day, one week, one month, two months, and three months after birth. One group of infants received two injections of poliomyelitis vaccine at three and four months of age and determinations of γ-globulin and antibodies were done at six or seven months of age.

The γ-globulin levels obtained in the mothers and at the various ages in the infants were similar to those reported in other studies. In general, the infants had the accepted normal values throughout the first six months of life.

Preliminary studies suggest that there is not a close correlation between the globulin levels and the antibody responses obtained.

130. Inulin and Free Water Clearance Studies in Potassium-Deficient Rats

MALCOLM HOLLIDAY; THOMAS EGAN,* and PATRICIA WIRTH,* Pittsburgh

It has been possible to produce in rats a histological lesion localized to the collecting tubule. The lesion, a result of potassium deficiency, has been associated with a diminished ability to concentrate urine. No evidence histologically of glomerular damage has been demonstrated in potassium deficiency, and in the present studies virtually no histologic alteration to the proximal tubule was observed. With so localized a lesion and with specific functional impairment, we felt it desirable to measure glomerular filtration rate, maximal urinary dilution, and free water clearance rates in potassium deficient and control rats. The potassium-deficient rats were fed a basal electrolyte deficient diet with supplemental sodium chloride for 28 to 33 days. The presently reported controls were fed standard laboratory chow. Weights of the two groups were comparable. Nine potassium-deficient and six control rats have been studied to date. Glomerular filtration rate, as measured by inulin clearance using a constant infusion technique, was reduced in the potassium-deficient rats by an average of 19%. Osmolar clearance rates were not significantly different. The solute concentration of the control group averaged 56 mOsm/L, that for the potassium deficient group 72 mOsm/L. The mean calculated free water clearance was reduced in the latter by 20%. If, however, this figure is expressed in terms of 100 cc. of glomerular filtration, no difference between the groups can be demonstrated.

The mechanism leading to reduction in glomerular filtration rate cannot be specifically defined from these studies. No glomerular changes were noted in the histologic sections. The hyperplasia of the collecting tubules observed in the sections is such that some tubular obstruction would appear to exist. Distended and dilated tubules in severe potassium deficiency provide further evidence that obstruction may exist. Such obstruction could account for the reduced filtration rate by increasing intraluminal pressure.

The influence of acute reduction in glomerular filtration rate on water diuresis with a concomitant reduction in osmolar excretion has been well defined. However, this effect would not appear directly operative here since osmolar excretion rates were not different. The authors would tentatively postulate that tubular obstruction would elevate tubular pressure, diminish the volume of fluid delivered to the site where dilute urine is formed, and thereby account for the diminished excretion rate of water. Alternate hypotheses may also account for these changes.

Studies on animals on a control diet and on animals following potassium repletion are in progress.

131. A Functional Enzymatic Deficiency of Cortisol Metabolism in Young Infants

GERALD H. HOLMAN * and CLAUDE J. MIGEON, Johns Hopkins

Cortisol, the major adrenocorticosteroid produced in man, is known to undergo certain metabolic processes prior to renal excretion. In adult subjects a very small amount is excreted as free cortisol, while much of the remainder is converted to dihydrocortisone and dihydrocortisol by specific liver dehydrogenases. These dihydro-compounds are further reduced by other liver enzymes to the tetrahydro-derivatives; they are then conjugated with glucuronic acid and excreted.

The daily urinary excretion of 17-hydroxycorticosteroids was determined in full term newborns, premature babies, and young infants. The free fraction and that released by β-glucuronidase hydrolysis were estimated separately. The ratio of glucuronide to free compounds (G/F) is equal to or greater than 10 in adults, but in full term newborns and some of the young prematures, it was close to 1.

When infants under one year of age were given ACTH-GEL intramuscularly (2-2.5 mg. per kilogram, q12h) the G/F ratio fell below the control values; this was particularly true in the premature group. This increased excretion of free steroids is possibly due to a defect in the reduction mechanism of cortisol and/or a deficient conjugation with glucuronic acid.

In an attempt to study this problem, cortisol was administered intravenously (2 mg. per kilogram); in each instance the G/F ratio in urine was found to be lower than that obtained following the intravenous administration of tetrahydrocortisone (2 mg. per kilogram). In addition, the G/F ratio in the tetrahydrocortisone experiments was

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lower in the newborns than in the older infants or adults.

The data suggests that there is a functional deficiency in the reduction mechanism of cortisol which improves as the child matures. In addition there is a defect in conjugation of the tetrahydroderivatives; this also improves with maturity. These deficiencies are more evident when the hormonal levels are raised either endogenously or exogenously.

132. Fetal Thyroid Development

ANTONE G. JACOBSON * and ROBERT L. BRENT,* Jefferson
Introduced by WALLACE W. MCCRORY.

A knowledge of the qualitative and quantitative aspects of fetal thyroid function is important because of (1) its basic contribution to the correlation of histology and physiology, and (2) the prevention of human fetal thyroid damage from administered I^{131} or I^{133} present in radioactive fallout.

The latter may be significant, because as yet no one knows which of the nuclear fallout isotopes has the smallest maximum tolerable dose. At the present time most investigators are concerning themselves with the bone-seeking isotopes. As yet no maximum tolerable dose for the fetus has been established; in fact, none has even been suggested.

Before anyone can establish such a maximum permissible dose in the fetus, a great deal more would have to be known about the physiological variability of the fetal thyroid at different stages of gestation.

In order to determine this, radioactive NaI^{131} was administered to pregnant mice at different stages of gestation. Twenty-four hours after injection the mother was anesthetized, and the fetuses were delivered by Caesarean section. The maternal thyroid, fetal thyroid and other fetal tissues including the placenta were dissected, weighed, and digested in NaOH. The radioactivity of the tissues was then determined in a scintillation-type well counter.

Because of the techniques utilized in counting the thyroid, the initial time for mouse fetal thyroid to pick up iodine was found to be earlier than has been previously reported in the literature.

1. Radioiodine uptake studies demonstrated that the mouse fetal thyroid accumulates iodine between the 14th and 15th day of gestation in mice with an average gestational period of 18½ days. The ability to concentrate iodine precedes by more than a day the formation of follicles and colloid.

2. The development of thyroid function with respect to iodine uptake is a gradual process.

3. The placenta plays a peculiar regulatory role in the fetal thyroid uptake.

The significance of these findings will be discussed with respect to the differential dose that can be expected to be received by the fetus when a dose is administered to the mother. An explanation is offered for some of the discrepancies of previous estimates of fetal thyroid function.

133. Changes in Acid-Base Balance and Blood Electrolytes in Vigorous and Depressed Newborn Infants

L. STANLEY JAMES,* Columbia
Introduced by RUSTIN MCINTOSH

The oxygen saturation, pH, carbon dioxide tension, buffer base, chloride, sodium, potassium, and lactic acid in blood samples from the umbilical artery and vein and serial samples from the aorta, left atrium, inferior vena cava, and portal vein, collected serially from 2 to 90 minutes after birth, have been studied in 50 normal and 10 depressed infants. The high carbon dioxide tension, low oxygen saturation, and low pH indicate that during the terminal phases of delivery and in the immediate neonatal period, during the onset of respiration, almost every infant shows biochemical effects of oxygen deprivation (asphyxia). In the depressed infants, not only was the carbon dioxide tension significantly higher and the pH lower than in the vigorous group but a superimposed metabolic acidosis and high potassium levels were also present presumably as a result of more prolonged asphyxia.

In the vigorous infants, who ventilate well, these biochemical changes rapidly readjust, but in the depressed or heavily sedated infants in whom lung expansion is poor and underventilation may persist for some time, the biochemical changes rapidly deteriorate, the pH falling as low as 6.7, and the P_{CO_2} rising to over 115 mm. Hg within minutes of delivery. The lactic acid levels change more slowly and appear to rise sharply in the first 10 to 15 minutes after birth. The significance of these changes in relation to cord blood levels will be discussed and the importance of such studies in the total evaluation of an anoxic insult to a newborn infant will be stressed.

134. Study of the Effect of Experimentally Induced Hypothyroidism on Antibody Production, Catabolism, and the Anamnestic Response

DOUGLAS E. JOHNSTONE and JOE W. HOWLAND,* University of Rochester

A state of complete hypothyroidism was induced in albino rabbits and dogs by repeated intravenous injections of large doses of radioactive iodine. Uptake of tracer doses of I^{131} and protein-

* By Invitation

bound iodine determinations were used to verify lack of normal thyroid function compared to normal control animals.

Each animal was given an equivalent dose of fresh washed sheep RBC. The changing concentration of antisheep RBC hemolysins was determined colorimetrically on decomplemented serum specimens obtained from time to time from normal and hypothyroid animals. When, after an initial rise in hemolysin titer, there occurred a marked reduction in antibody concentration, each group of animals was divided into two groups. Half was given a second or booster dose to study the anamnestic response. The remainder were given infusions of homologous sera containing a high concentration of antisheep RBC hemolysins in order to study the rate of catabolism of these antibodies in normal and hypothyroid animals.

The rate of rise and fall of antibody concentration after initial injections of washed sheep RBC was found to differ in the two groups in that the hypothyroid animals had a significantly longer persistence of antibodies than normal animals. Similarly the anamnestic responses differed in that hypothyroid animals had a more marked rise in antibodies than normal animals. The rate of catabolism of passively infused antibodies in the two groups was most striking. Whereas the half life of antibodies in normal animals was invariably approximately two to three days, regardless of amount of antibody infused, the half life or decay rate in hypothyroid animals was markedly prolonged and varied from 10 to 38 days, varying roughly proportionally to the concentration of passively infused antibodies.

135. Experimental Salicylate Poisoning: Effects of a Carbonic Anhydrase Inhibitor and Bicarbonate

S. A. KAPLAN; A. YUCEOGLU *; J. STRAUSS,* and F. T. DEL CARMEN,* State University of New York, Brooklyn

Since no effective antidote is known to counteract the effects of poisoning by salicylates, effective therapy must be directed at the promotion of elimination of the poison. Salicylates are eliminated largely by the kidneys but, because of protein binding and other limiting factors, their rate of excretion is slow.

The experiments to be reported demonstrate the effectiveness of the carbonic anhydrase inhibitor acetazoleamide (Diamox) in promoting the excretion of salicylates in dogs to which salicylates were administered. The renal excretion was increased up to ten fold following administration of acetazoleamide especially when the animals were first made acidotic by ammonium chloride administration. These changes occurred without appreciable change in glomerular filtration rate indicating that acetazoleamide exerts its effects on the renal tubular reabsorptive mechanism.

The mechanism of this increased excretion appears to be related to the fact that salicylate, which is a weak anion, diffuses more rapidly into an alkaline urine in very much the same way as the weak base ammonia diffuses more rapidly into an acid urine. Acetazoleamide, by inhibiting the action of renal carbonic anhydrase and, therefore, the secretion of hydrogen ions, produces conditions favorable for the excretion of salicylates. However, this drug, by inhibiting the action of carbonic anhydrase, also impairs the primary defense mechanism of the body against acidosis, and its use could be dangerous, since metabolic acidosis is an outstanding feature of the deranged metabolism which occurs in salicylate poisoning.

A further series of experiments was therefore carried out on rats which received larger doses of salicylates than were given to the dogs, doses which approached the lethal level. The experiments confirmed the fear that the beneficial effects derived from acetazoleamide (increased salicyluria) were considerably outweighed by the harmful effects (impaired defense against acidosis). Seven hundred milligrams of salicylate per kilogram body weight was found to produce severe prostration and hyperventilation when administered intraperitoneally to rats. All the animals recovered, however. The concomitant administration of acetazoleamide and salicylate, on the other hand, was followed by a marked increase in mortality. The dose of salicylate in these latter experiments was also 700 mg. per kilogram, and acetazoleamide was administered in dosage varying from 5 to 20 mg. per kilogram.

The administration of sodium bicarbonate in addition to salicylate was found to protect the animals to a significant degree against the action of acetazoleamide. Also, in a further series of experiments it was found that administration of bicarbonate did not increase the mortality of animals receiving injections of salicylates simultaneously but no acetazoleamide. This was interpreted as evidence against the frequently stated clinical impression that administration of bicarbonate increases the toxicity of salicylates in the early stages of salicylate poisoning.

On the basis of the above findings, it is recommended that acetazoleamide should never be used in the treatment of poisoning with salicylate. On the other hand, it appears that the administration of sodium bicarbonate to patients who are in the age group where acidosis is especially prone to occur (up to three years) is warranted.

* By Invitation

136. Lactic-Acid Milk in Feeding of Premature Infants

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The first part of the study involved 44 premature infants of birth weights ranging from 1400-1800 gm. The infants were placed in one of three isocaloric feeding groups, as follows.

Group 1. consisted of 18 infants fed a half-skimmed cow's milk mixture (Alacta).

Group 2. consisted of 14 infants fed a lactic-acid milk mixture (Pelargon).

Group 3. consisted of 12 infants fed an evaporated milk mixture.

It was observed that the infants fed the lactic-acid milk 1. gained significantly less weight than those in other groups; 2. had higher morbidity, and 3. frequently developed pallor.

In the second part of the study we investigated the blood pH and CO_2 content of premature infants fed the above three milk mixtures. Infants fed the lactic-acid milk mixture had a lower blood pH and CO_2 content than infants fed the other two milk mixtures.

The lactic-acid milk fed in the study was prepared by the addition of U. S. P. lactic acid which is a racemic mixture of the *d* and *l* forms. It is known that the *d* form of lactic-acid is not well metabolized by the liver. This and other aspects of lactic-acid milk feedings for prematures will be discussed.

137. Serologic Response to Asian Influenza in Children Following Infection and Vaccination

D. T. KARZON; R. H. BUSSELL*; L. MOSOVICH,* and D. L. KING,* Buffalo

Although infants and children are known to have a high incidence of infection with influenza virus, the attending clinical and serological response has not been well documented. The 1957 outbreak of Asian influenza provided an opportunity to observe the behavior of a new antigenic variety of influenza both as a natural infection and as a vaccine. A group of 78 children, 3 months to 6 years of age, was immunized with monovalent Asian influenza vaccine (200 units per milliliter) in accordance with the schedule of the American Academy of Pediatrics; two injections one week apart, either 0.1 ml. subcutaneously or intradermally, or 0.5 ml. subcutaneously. The following observations were made: 1. The antibody response to vaccine was approximately equal, independent of the route used. The overall effectiveness as measured by hemagglutination-inhibition (HI) response was approximately 70%. Negligible toxicity was noted. 2. More than ⅓ of the children had serologic evidence of infection with Asian influenza virus prior to the initial vaccination. Of these, 60% had a history of mild upper respiratory infection, usually febrile, and often associated with a cough and sore throat. Of great interest was the 40% who experienced no illness known to the family and who therefore were examples of inapparent infection.

The serologic response to natural Asian influenza infection in hospitalized and nonhospitalized children was compared to the vaccinated group. The following data became evident: 1. Complement-fixing (CF) antibody was measured against the viral antigen (V antigen of Henle). CF antibody was produced regularly following influenza infection but only to a limited degree both in number of individuals and in titer following vaccination. 2. The HI response occurred following either infection or vaccination.

The effect of past serologic experience with A prime virus upon CF and HI antibody response as measured using Asian and A prime antigens will be presented.

* By Invitation

138. Studies of Unesterified Fatty Acid Metabolism in Infants

ROBERT KAYE and MITIO KUMAGAI,* Pennsylvania

Previous work in this laboratory has shown that the resistance to fasting ketosis in young infants is not a reflection of an increased rate of utilization or renal excretion of ketone bodies.

Recently Gordon has shown that the lipid fraction chiefly responsible for furnishing the energy utilized during fasting is the unesterified fatty acid (U. F. A.) of the plasma. We had previously shown that infusion of neutral fat intravenously resulted in less ketonemia in young as compared to older infants. Nevertheless, it seemed important to determine whether or not differences in availability of the metabolically active ketone precursor, U. F. A., might explain observed differences in ketonemia susceptibility.

Determinations of U. F. A. concentration have been carried out in 39 infants aged 8 hours to 17 months. Fasting levels have averaged 0.55 mEq. per liter in eight infants over three months, 0.45 mEq. per liter in six infants over three months, and 0.81 mEq. per liter in eight infants from 8 to 36 hours after birth. Simultaneous ketone determinations in the newborn infants exhibited the previously observed low values (Av. 4.3 mg. %).

The depressing effect of feeding on U. F. A. concentration has been noted in 17 infants, whose mean value was 0.37 mEq. per liter.

The expected fall, 58%, was noted in eight infants after glucose ingestion. Epinephrine injec-

tion in eight infants resulted in an average increase of 108% over the initial values.

The results indicate that infants under three months of age are capable of mobilizing U. F. A. and that the resistance to ketosis in this age group is probably the result of decreased ketogenesis by the liver rather than of inability to mobilize lipids.

139. The Plasma Potassium Concentration in Normal Premature Infants

HANS G. KEITEL* and JOYCELYN BLAKELY,*
Jefferson and Howard
Introduced by JAMES AREY.

The majority of published data indicate that the potassium concentration of plasma of infants in the neonatal period is elevated, although some reports indicate that low values may be found quite frequently. This problem was reexamined because analytical factors, the composition of the food ingested, and the pH of the blood influence the concentration of potassium in plasma; many of these variables are not known to have been controlled in the earlier reports.

Blood was collected in plain and in siliconized glassware and was centrifuged immediately and at varying intervals after the blood was removed in order to observe the possible effect of platelet lysis and the effect of standing on the plasma potassium concentration. The plasma hemoglobin concentration was determined routinely to observe the (maximal) effect of hemolysis on the plasma potassium concentration. The data indicate that blood collected in siliconized glassware and centrifuged within 15 minutes yields plasma potassium values that represent the in vivo status. Over 400 blood samples, obtained from 100 infants weighing from 2 to 5½ lb., were handled to avoid extra vascular changes (vide supra), and the results described below were obtained.

The plasma potassium concentration in premature infants during the first few hours of life ranged between 3.8 to 5.2 mEq. per liter; this is within the range of normal for fasting adults (4.2 mEq. per liter; S. D. 0.25). After the first day of life, the average potassium concentration rose to 5.3 mEq. per liter and could be made to vary from 3-4.5 mEq. per liter (for example, after glucose feedings) to 7 to 8 mEq. per liter (for example after 4 to 6 mEq. of potassium per kilogram had been ingested per 24 hours). There was not indication that the potassium concentration of plasma in infants weighing from 2 to 4 lb. differed from that of premature infants weighing more than 4 lb.

The addition of sodium bicarbonate (2 to 3 mEq. per kilogram per day) to a standard formula resulted in a decrease in the plasma potassium concentration and correction of the mild metabolic acidosis which is frequently seen in infants.

The data can be interpreted as indicating that the concentration of potassium in the plasma of premature infants is substantially the same as that of normal fasting adults and is subject to the same variables if the plasma potassium concentration of the infants is corrected for the 0.1 to 0.2 lower pH that they have compared to normal fasting adult subjects and if the composition of the diet and the time the blood sample is removed following eating are taken into consideration.

140. Incidence and Persistence of Poliovirus in the Oropharynx of Children with Acute Poliomyelitis

SIDNEY KIBRICK; AUGUSTO SCHUSTER,* and JOHN F. ENDERS,* with the Assistance of Eleanor R. Kaufman, Harvard

Daily throat swabbings were obtained from 50 patients admitted to The Children's Medical Center in 1955 with the diagnosis of acute poliomyelitis. All of the specimens were tested in human amnion-cell cultures, and those from 37 of the patients were tested as well in cultures of monkey renal tissue for poliovirus. Single fecal specimens from 40 of the children, obtained during the first week of hospitalization, were also tested in monkey renal tissue culture.

Poliovirus Type I was recovered from the oropharyngeal swabbings, feces, or both, of 42 of the patients. In 13 of this group, the virus was demonstrated in the oropharynx from 8 to 17 days after onset of their illness, and in 6 of the 13 this agent was recovered after the seventh hospital day.

The total number of positive oropharyngeal cultures dropped sharply as hospitalization progressed. Thus, of the 88 cultures positive for this agent, 72 were obtained during the first four days of hospitalization, 19 during the next three days, and only 7 subsequently.

For 17 of the patients the titer of virus in an aliquot of the first positive oropharyngeal specimen was determined in cultures of human amnion cells. In most instances only small amounts were detected.

The low concentration of this agent in the oropharynx and its rapid disappearance from this site during hospitalization may in part account for the infrequent occurrence of contact infections among hospital personnel concerned with the care of poliomyelitis patients.

From the oropharyngeal swabbings of nine of the patients a total of 58 additional agents were recovered. Of these, 36 were isolated in amnion-cell cultures, 7 in monkey renal cells, and 15 in cultures of both tissues. Certain of these agents have been identified as adenoviruses, as Coxsackie

* By Invitation

B2, and as croup-associated Viruses. Since, in most instances, these viruses were encountered several days after admission of the patients, they presumably became infected during hospitalization.

141. Blood Volume Studies in Infants and Children as Determined by Cr^{51}

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KAMTHORN SUKAROCHANO*;
LUCIO PARENZAN,* Pittsburgh
Introduced by STUART S. STEVENSON.

Determination of blood volume in the pediatric surgical patient has become of increasing importance. Weighed sponges, weighed patients, or estimates by the operating team have major deficiencies, especially in the newborn infant. The adult method of tagging red cells with Cr^{51} seemed to offer possibilities, if accuracy and safety could be assured.

Our first efforts were directed toward rabbits and dogs in order to work out a modification of the adult procedure that would be feasible in the infant. Six rabbits and ten dogs were studied by determining red cell volumes, then bleeding a known amount and redetermining the volume. Wide variations in results occurred. Seven splenectomized dogs were studied, as we felt that the error lay in the spleen's response to bleeding. This proved to be true as the pre- and post-bleeding results were checked to 0.3-3.1% error. A simplified method was devised during these animal experiments and tried out in a number of in vitro determinations. When these were checked to 1.9-2.9% error, we concluded that we had a useable method.

Application of the method was made to children according to age groups: 1-7 days, 7 days-3 months, 3 months-1 year, 1-2 years, 2-4 years, 4-6 years, 6-8 years, and 8-12 years. Ten patients were studied in each group. They were studied preoperatively and, in most cases, again postoperatively. The operating team estimated blood loss by usual techniques, and this was checked against the postoperative determination. By these studies it became possible to arrive at standard cubic centimeter per kilogram values for children in each age group. The results were as follows.

* By Invitation

	Packed Red Cells	Blood Volume
1-7 days	33.0 cc/Kg.	81.0 cc/Kg.
1 wk.-3 mo.	30.8 cc/Kg.	78.5 cc/Kg.
3 mo.-1 yr.	24.5 cc/Kg.	73.6 cc/Kg.
1 yr.-2 yr.	23.7 cc/Kg.	65.8 cc/Kg.
2 yr.-4 yr.	23.3 cc/Kg.	66.9 cc/Kg.
4 yr.-6 yr.	25.9 cc/Kg.	65.2 cc/Kg.
6 yr.-8 yr.	26.3 cc/Kg.	69.8 cc/Kg.
8 yr.-12 yr.	25.1 cc/Kg.	63.7 cc/Kg.

It soon became apparent that, although the method was accurate, useable, and safe, it was cumbersome and time-consuming. It took an hour to an hour and one-half to get a reading with the spinning-down techniques employed. A coil of measured volume was wrapped around a tube and placed in the counter which in turn was placed in the operating room. Whole blood was drawn directly from the patient into the counter after appropriate labelling of cells in the circulation. By proper calibration and interpolation it was possible to get rapid and accurate results.

It is obvious that such a method as this (slow or rapid) has many applications in clinical pediatrics, and it is for this reason its details will be presented.

142. The Effect of Cortisone and ACTH on Chromium^{51} Red Blood Survival in Erythroblastosis Fetalis

WILLIAM KRIVIT,* University of Minnesota
Introduced by ROBERT A. GOOD.

The beneficial effect of steroids in acquired hemolytic anemias has prompted similar therapy in erythroblastosis fetalis. Klingberg and Geppert have presented evidence for amelioration of the clinical process by use of cortisone and ACTH.

This study was devised to analyze the effect of steroids on the survival of red blood cells in erythroblastosis fetalis. The individual infant's autogenous red cell survival was measured by the chromium^{51} method.

The results in the beginning of the study indicated that there was a correlation between red cell Cr^{51} survival and clinical severity.

Cortisone therapy (6 mg. q 6 hr.) was begun in five infants after the red cell Cr^{51} survival was established. There was no significant change in Cr^{51} survival curve that could be attributed to cortisone.

In a small controlled series (seven with cortisone and six without), cortisone therapy was begun immediately after exchange transfusion. There was no significant difference in the red blood cell Cr^{51} survival rate between the control or cortisone group.

The red blood cell Cr^{51} survival rate did not change after the cortisone was discontinued in three infants.

ACTH 12.5 mg. b. i. d. did not affect the Cr^{51} survival curve in one infant.

The survival of Rh— cells was also studied in postexchange erythroblastosis fetalis. A rate of destruction of these normal Rh— cells greater than in normal infants was noted. Cortisone did not affect the survival of Rh— cells.

The conclusion is that in the immediate post-exchange period, steroid therapy does not produce a significant change in rate of Cr^{51} red blood cell

survival in erythroblastosis fetalis of either Rh+ or Rh− cells.

143. Electron Microscope Observations on the Human Fetal Adrenal

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The human fetal adrenal gland has an unusual developmental pattern. The gland is strikingly large and is 80% composed of a central reticular zone known as the fetal zone. A surrounding layer consisting of a poorly differentiated glomerular zone and a thin fascicular zone is known as the adult cortex. The fetal zone involutes rapidly after birth, and simultaneously the adult cortex differentiates and grows.

Observations on the fetal adrenal with the electron microscope have revealed several unusual features. Cells of the fetal zone possess an extensive network of tortuous, anastomosing tubules. These resemble the agranular ("smooth") endoplasmic reticulum that has been described in other cells, but fetal zone cells are unique in the enormous extent to which this network is developed. The agranular endoplasmic reticulum is believed to provide an extensive communication system between various intracellular structures. In the fetal zone, we have observed direct communications with the granular ("rough") endoplasmic reticulum, but thus far, not with other structures. Palade has shown that the agranular endoplasmic reticulum is found to predominate in cells active in lipid metabolism, which correlates with the high steroid biogenetic capacity of fetal zone cells which we have observed in previous studies.

The granular endoplasmic reticulum is present in moderate quantity in fetal zone cells. These lamellar structures consist of a series of membrane-bounded flattened cisterns with frequent communications between adjacent layers. Particles on the outer surface of the membranes, which are responsible for its rough appearance, have been identified by others as ribonucleoproteins; these compounds are involved in the process of protein synthesis.

The mitochondria of fetal zone cells are numerous, widely variable in size, and lack the usual orderly arrangement of cristae seen in mitochondria of other mammalian cells. Instead, a series of fine uniformly spaced tubules are compressed into various portions of the mitochondria.

Cells from the adult cortex are strikingly different from those of the fetal zone. Cytoplasm is scanty and lacks extensive structural development, suggesting a metabolically less active tissue. These anatomical differences may reflect the physiologic needs of the fetus. The glomerular zone is believed to be concerned with electrolyte homeostasis, for which the fetus can depend on the mother. We believe that the poor differentiation of the glomerular zone in the fetus, together with its rapid differentiation after birth, reflects the physiologic demands which this tissue must meet.

* By Invitation

144. Familial Pernicious Anemia in Children

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Introduced by ROBERT H. PARROTT.

Recently we have observed and studied two siblings with macrocytic anemia who did not fit the criteria for the diagnosis of megaloblastic anemia of infancy. Investigations were conducted to more accurately delimit their difficulty. This included vitamin B_{12} blood assay and radioactive cobalt B_{12} absorption studies. These revealed findings consistent with pernicious anemia. Intestinal absorption studies including xylose tolerance tests, fat and vitamin A tolerance tests as well as stool examinations were conducted. These excluded a "malabsorption syndrome." Gastric function studies and a gastric biopsy revealed adequate structure and function. These findings and their implications will be discussed.

145. Effect of Convulsions on the Blood-Cerebrospinal Fluid Barrier and on Cerebrospinal Fluid Levels of Glutamic Oxalacetic Transaminase and Lactic Dehydrogenase

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Introduced by JONATHAN T. LANMAN.

Prolonged convulsions in puppies increased the permeability of the blood-cerobrospinal fluid barrier and raised the cerebrospinal fluid (CSF) levels of glutamic oxalacetic transaminase (GOT) and lactic dehydrogenase (LDH).

Convulsions were produced in puppies 2 to 6 months of age either by the intravenous administration of metrazol or by the application of electric shock, using electrodes placed over the parietal region of the skull. Controls consisted of puppies of similar ages and weights treated with a sham procedure.

The effect of convulsions on the permeability of the blood-cerebrospinal fluid barrier of puppies was studied by measuring the rate of passage of radioactive iodinated human serum albumin (RIHSA) from the blood to the CSF. RIHSA (11μc. per kilogram) was injected and then convulsions were produced immediately for variable periods of time by one of the two above methods. At the cessation of the convulsive period, or a similar period of time in the control animals,

Type of Experiment	Puppies, No.	Concentration Ratio × 10^{-4} Range	Av.
Control	28	10.1 - 61	27.1
Metrazol seizures	16	27.2 - 2240	421.8
Electroshock seizures	14	58.0 - 2378	500.4

samples of CSF (cisternal) and plasma were obtained. Radioactivity was measured in the well type scintillation counter and recorded as counts per milliliter per minute. Concentration ratios (cisternal fluid radioactivity/plasma radioactivity) were determined in the three groups of puppies (control, metrazol, and electroshock).

The following results were obtained in animals with convulsions of more than 30 minutes duration and their controls.

When convulsions were prolonged for more than 30 minutes the concentration ratio was significantly higher than the controls. When convulsions were of shorter duration (less than 30 minutes) the concentration ratios were not significantly different from the control group. Previously we have demonstrated similar changes in the rate of passage of albumin from plasma into cisternal fluid with prolonged hypoxia, and we believe such changes reflect alterations in the permeability of the blood-CSF barrier to albumin.

GOT and LDH levels of CSF following convulsions were measured using the methods of Karmen et al., and Wroblewski and La Due, respectively.

The following results were obtained.

Puppies with convulsions showed increased CSF levels of GOT and LDH as compared to the controls. Most puppies with convulsions with an increased concentration ratio of radioiodinated albumin also had an elevation of the cerebrospinal fluid GOT and LDH. This suggests that the increase in GOT and LDH following convulsions may be due in part to an increased permeability of the blood-cerebrospinal fluid barrier to these enzymes.

146. Studies of Cardiac Rate in Newborn Infants

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Because of the variability in cardiac rate noted clinically in newborn infants, a study was undertaken to record systematically cardiac rate in ten infants under a variety of physiologic conditions. An average of 47 observations per infant comprise the major part of the data. The observations were made in relationship to feeding, motor activity, sucking activity, and stimuli such as brushing of the lips and abdomen.

A detailed analysis of changes was made by recording rates during 30-second prestimulus, stimulus, and poststimulus periods. Motor activity was rated quantitatively. The infants were observed within one hour before or after feedings.

Some of the findings which will be discussed are the following.

1. A consistently significant correlation between the level of prestimulus heart rate and the degree of response to stimulation. The lower the rate before stimulation, the greater the response to the stimulus. These data confirm the "law of initial values" of Wilder and Lacey derived from studies of older subjects.

2. More rapid heart rate prior to feedings.

3. Differences in response depend upon state of satiation.

4. High correlation between heart rate and motor activity responses.

5. Sequential "see-saw" responses to the same stimulus. (When stimulated at a high base line, cardiac rate was observed to decrease and remain

* By Invitation

Type of Experiment	GOT Units/Ml/Min. Puppies, No.	Range	Av.	LDH Units/Ml/Min. Puppies, No.	Range	Av.
Control	14	1.0 - 3.25	1.93	15	1.11 - 7.78	4.68
Metrazol seizures	15	2.3 - 20	7.18	17	4.44 - 39.4	14.85
Electroshock seizures	13	4.25 - 12	8.6	9	5.56 - 16.7	11.4

low. The next stimulus would result in a significant elevation of rate.)

6. An analysis of the poststimulus periods indicated rather marked responses to the cessation of stimuli.

7. Extremely low or high cardiac rates which previously may have been considered to be clinically abnormal were noted to be physiologic.

An additional group of 10 infants was studied during sleep to note the relationship of EEG and heart rate patterns during stimulation. These findings indicate corticoautonomic relationships during sleep as manifested by changes in the EEG and cardiac rate patterns in response to stimulation during the sleeping state.

These studies indicate that cardiac rate in the newborn should be evaluated in relationship to physiologic state.

147. The Chloride of Bone

CHARLES C. LOBECK,* University of Rochester
Introduced by GILBERT B. FORBES.

It is postulated that the chloride present in bone is not in the crystals or the cells but located in the extracellular fluid. For this reason the chloride space of bone is frequently used as an estimate of its extracellular fluid volume. It has been observed, however, by several investigators that in dogs and rats the chloride space is sometimes larger than the actual water content of the bone. This study was designed to further investigate these observations.

The long bones of cats and rats, from which the marrow had been removed, were analyzed for chloride, ash, and water content. It was found that in the cat there was more chloride than would be predicted if the entire bone water content was an ultrafiltrate of plasma. The amount of excess chloride present was constant in all bones analyzed regardless of the water or ash content. To determine the exchangeability of the chloride some of the cats were injected with Br^{82} and after a period of two hours bones and blood removed for counting. It was observed, in bones of low water content, that the total bone chloride did not exchange completely with plasma chloride. However, in bones of higher water content the exchangeability was 100%.

The studies of the bones of rats revealed an excess of chloride in young animals, but in animals older than approximately 100 days the chloride space was equal to or less than the bone water content. The amount of excess chloride varied directly with the water content and inversely with the ash content of the bones.

On the basis of these studies it was concluded that the chloride space is not a valid estimate of the extracellular fluid phase of bone in cats or young rats. The location of the excess chloride of bone is not known, but it was found in cat bones of low water content that the total chloride of the bone was as little as 50% exchangeable after two hours. This may indicate, if the excess chloride is located in the collagen, that the extracellular fluid phase of bone in older animals is not in direct contact with some of the organic matrix. In young rats it is less likely that the excess is in the organic phase but more likely that it is in the fluid phase.

148. Observations on Transfer of Vitamin B_{12} from Mother to Fetus and Newborn

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Introduced by LOUIS K. DIAMOND.

These experiments were designed to determine the range and kinetics of placental transfer of vitamin B_{12} in the human. Observations were also made on the range and mechanism of vitamin B_{12} excretion into breast milk and its gastrointestinal absorption in the newborn.

Materials and methods employed included small tracer doses of $Co^{60}B_{12}$, half-life; 72 days. Hepatic uptake of radioactivity in mother and newborn by a probe-type scintillation counter was employed to estimate retention and transfer of administered $Co^{60}B_{12}$. Determination of radioactivity in aliquots of placental homogenates, colostrum, breast milk, and blood were performed in a well-type scintillation counter.

Results showed that the transfer of single physiological doses of $Co^{60}B_{12}$ to the human infant via the placenta varied from 0.3% to 28.0% of the dose administered to the mother. Substantial amounts were not transferred until 24 hours after administration. Maximum transfer occurred within 15 to 21 days and increased no further thereafter. After oral administration, smaller amounts were found in those infants whose mothers had poorer intestinal absorption. Deposition of $Co^{60}B_{12}$ in the human placenta varied from 0.2% to 6.1% depending upon the time of antepartum administration. The range after maximum placental transfer occurred was 1.5% to 2.0%. Over 90% of the latter $Co^{60}B_{12}$ resided in the placental tissue rather than the placental blood.

Concentrations of $Co^{60}B_{12}$ per milliliter in breast milk following I. M. injection was of the order of 10^{-6} of the administered dose. Excretion by the lactating breast continued to increase into the sixth day although the blood level of $Co^{60}B_{12}$ reached its peak in 18 to 30 hours. Concentrations in colostrum were 6 to 17.5 times that of milk.

The absorption of 0.05-0.1 μg. doses of $Co^{60}B_{12}$ in the newborn after oral administration was found to

* By Invitation

be 75% to 95% of the dose. Intestinal absorption of 0.5 to 0.9μg. doses during last trimester of pregnancy was 27% to 70%.

These studies may lead to a clearer understanding of the reasons for the decline in maternal serum vitamin B_{12} levels during pregnancy and for the high serum B_{12} levels found in the fetus.

149. A Study of the Coagulation Properties of Infants' Platelets

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Introduced by ROBERT H. HIGH.

Since deficiencies of blood coagulation factors are known to exist in the neonatal period, a study of the properties of platelets obtained from newborn infants has been carried out.

Platelets were obtained from infants during the course of exchange transfusions performed for hemolytic disease of the newborn. Although the platelets are often low in number in such infants, they should be qualitatively representative of platelets from normal newborn infants. The first 40 ml. of blood were withdrawn during the exchange transfusion prior to giving donor blood to the infant. Two subsequent samples of 20 ml. each were collected; prior to each of these, the infant received 20 ml. of donor blood. Platelets were obtained from the entire 80 ml. by centrifugation. These were then washed three times and suspended in such an amount of saline as to yield a final concentration of platelets between 180,000 and 280,000/cu. mm. Platelets from adults were obtained from 100 ml. of normal adult blood and were similarly prepared. The number of platelets in paired adult and infant specimens did not vary more than 40,000/cu. ml.

The platelet functions were measured by their effect upon the recalcification time and on the thrombin time of platelet-poor plasma (Factor III) and by utilizing the platelets in the thromboplastin generation test with normal plasma and serum (platelet thromboplastic factor).

Infants' platelets are as effective as are those of adults as measured by their effect upon the recalcification time and upon the thromboplastin generation test. The degree of correction produced in the thrombin time suggests a mild deficiency in factor III but this is not statistically significant. Preliminary studies of fractionated Factor III will be presented.

Crude tests of clot retraction fail to demonstrate any abnormality of infants' platelets.

* By Invitation

150. The Significance of Bone Ages in a Group of Children Referred to an Endocrine Clinic

WILLIAM J. MELLMAN *; ALFRED M. BONGIOVANNI, and JOHN HOPE,* Pennsylvania

Bone age surveys of 131 patients who were referred to the endocrine clinic have been reviewed. Multiple ossification centers have been studied using standards compiled by Stuart, Stevenson, and Maresh.

Of 83 children who were examined because of short stature or delayed bone age, 37 were retarded more than 30%. Of this latter group about 25% were considered constitutional, and another 25% were proven to have hypothyroidism. Most of the remainder were suffering from nonspecific disease or nonendocrine metabolic disorders. Several children of constitutionally diminished stature with approximately normal bone ages are included.

Of 48 children with advanced bone ages, 54% were accelerated more than 30%. Of the latter group, 40% were believed to have constitutional sexual precocity and 40% have congenital adrenal hyperplasia. Whereas, in congenital hypothyroidism the bone ages of the youngest patients were significantly retarded, in congenital adrenal hyperplasia advancement of bone age was not generally present in the early months of life.

In seven children with gonadal dysgenesis, the stature was relatively more retarded than the bone age. In congenital adrenal hyperplasia, the bone age was relatively more advanced than the height.

Delayed bone age does not necessarily imply endocrinologic disease. The relationship of the time of diagnosis and treatment of the bone age of children with true endocrine disorders will be detailed.

151. Effects of Antimetabolites on Development of the Fetal Rat

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Four major classes of antimetabolites interfering in different ways in the synthesis of precursors of nucleic acid were studied for their effects on the development of the rat fetus. Compounds used included the following. 1. Folic acid antagonists, aminopterin and amethopterin which interfere with the conversion of folic acid to its formyl derivative, citrovorum factor, which here supplies single carbon fragments for the synthesis of purine (2 and 8 positions in the ring) and pyrimidine (5 methyl group of thymine) components of nucleic acid. These effects can be prevented by citrovorum factor.

2. The purine analogues 6-mercaptopurine, thioguanine, (2-amino-6-mercaptopurine), and 6-chloropurine which appear to interfere with the incorporation of preformed purines into nucleic

acids. In some animal tumors these effects have been prevented by administration of adenine, guanine, hypoxanthine, xanthine or adenylic acid.

3. The glutamine antagonists azaserine (O-diazoacetyl-L-serine) and DON (6-diazo-5-oxo-L-norleucine) which inhibit the conversion of formylglycineamide ribotide to formylglycine amidine ribotide (incorporation of N into the 3 position of the purine ring). In some systems the effects of these compounds may be prevented by adenine or inosine.

4. The 5-fluoro-substituted derivatives of uracil, uridine, deoxyuridine and orotic acid interfere with the conversion of a uracil containing compound to a thymine containing compound. Thymidine and uracil are protective against these drugs in some systems.

Groups of pregnant Wistar rats of known (within 12 hours) breeding date received single intraperitoneal injections of the test chemicals on one day (8th through 14th) of the gestation period. The rats were killed on the 21st day. The criteria of their effects at various stages of development were 1. the ratio between maternal and litter MLD_{100}; 2. teratogenic effects at doses permitting survival; 3. period of gestation when fetal susceptibility to each drug was most evident, and 4. protection by various metabolites against the lethal and teratogenic effects of the drugs on the fetus. Using 12 days of gestation as a base line azaserine caused complete fetal resorption at 1/40 the maternal LD_{50}, DON at 1/500, aminopterin at 1/12, and 5-fluorouracil at 1/4th. All compounds except aminopterin and DON caused gross abnormalities at sublethal doses. The effects of these antimetabolites on the 12 day fetuses differed in several ways. Teratogenic effects for each compound varied with the time of treatment. Whereas after the 12th day, the fetal toxicity of azaserine, DON, and aminopterin fell off sharply, it was not appreciably modified with the 5-fluorinated pyrimidines. Adenine and inosine gave some protection against the lethality of azaserine and DON. Although resorptions without teratogenic effects had been seen with DON alone, DON in combination with adenine caused abnormalities similar to those seen with azaserine. Citrovorum factor protected against the effects of aminopterin but adenine was not protective against 6-mercaptopurine.

Examples of the specific developmental abnormalities such as cleft palates, hare lips, sirenomelus, etc. produced by these different antimetabolites, similar within the groups, but different among the groups, will be shown.

152. The Blood-Brain Barrier for Bilirubin During Early Infancy

MARIDEE NASRALLA*; EVA GAWRONSKA,* and DAVID YI-YUNG HSIA, Children's Memorial Hospital, Chicago

The present paper describes some observations on the effect of age and birth weight upon the blood-brain barrier for bilirubin during early infancy. Simultaneous samples of serum and spinal fluid were obtained from 100 newborns. In each instance, the serum bilirubin was determined by the micro method described by Hsia et al. The spinal fluid bilirubin was determined (1) by direct reading in the Beckman DU Spectrophotometer at 453μ, and (2) by treatment with diazo reagent, and reading after thirty minutes at 540μ.

The results were expressed as a ratio of serum bilirubin/spinal fluid bilirubin. In our experience, the ratio in adults and older children remains at about 100. In young infants, the following ratios were obtained.

The serum bilirubin/spinal fluid bilirubin ratios in infants with erythroblastosis fetalis were not significantly different from those seen in normal newborns.

These data suggest that there is an increase in the permeability of the blood-brain barrier for bilirubin during early infancy. This appears to be most marked in the small premature. As the infant grows older, the blood-brain barrier becomes increasingly effective in preventing bilirubin from entering the spinal fluid.

* By Invitation

Weight			Age, Hr.			
			0-48	49-96	97-144	144-212
Less than 5 lb.	No.	Tested	10	20	13	6
		Mean	18.8	21.9	26.9	27.3
		S. D.	±10.6	± 7.5	±11.7	± 8.5
5 lb. 1 oz. to 7 lb.	No.	Tested	17	10	4	0
		Mean	22.6	35.6	41.7	—
		S. D.	±16.2	±16.8	± 9.0	—
7 lb. 1 oz. or more	No.	Tested	11	7	2	0
		Mean	43.9	48.8	33.3	—
		S. D.	±30.0	±25.9	±10.9	—

The significance of this in the pathogenesis of kernicterus in premature infants and those with erythroblastosis fetalis will be discussed.

153. Maturation of Renal Tubular Function Demonstrated in Slices of Kidney

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Maturation of renal tubular function, previously demonstrated in vivo by various techniques, has been studied in vitro utilizing the method of Gross and Taggart (Am. J. Physiol. 161:181, 1950). Intracellular accumulation of para-aminohippuric acid (PAH) and chlorphenol red (CPR) was measured in slices of kidney obtained from rabbits ranging in age from 27 days gestation to adulthood. PAH was determined colorimetrically in slice and medium, and CPR was visualized microscopically following 60 minutes of incubation in an atmosphere of oxygen. Oxygen consumption was measured manometrically.

Results reveal that PAH and CPR accumulation in slices of kidney remains at a low level from six hours postnatally to eight days of age, while slices from fetal animals near term accumulate more PAH than those from the neonatal group; adult levels are attained in slices from animals ten days and older. When PAH accumulation is based on nitrogen content or the dry weight of the slice rather than wet weight, the difference between the neonatal and fetal group is exaggerated and the PAH accumulation by slices of fetal kidney is identical with that of the adult. This similarity of fetal and adult slices is in part a result of rising nitrogen content in the slices with age. That the PAH accumulation observed was an active process and not diffusion, was confirmed by including flasks containing 5×10^{-5} M. dinitrophenol (DNP), a potent inhibitor of PAH transport, in each group. There was negligible accumulation in slices incubated with DPN when compared to those incubated without DNP. When oxygen consumption is based on nitrogen content of the slice, the slices from the newborn to the 9-day-old group which showed the least PAH accumulation also show the least oxygen consumption, while the fetal slice consumes almost as much oxygen as the adult per milligram of nitrogen. In all age groups, except fetal rabbit, in which there was a significant PAH uptake by slices of kidney, CPR was visualized in renal tubules microscopically. In unstained fresh smash preparations, dye appears as a red substance in juxtaglomerular tubular calls.

In an attempt to establish a biochemical basis for the observed differences in accumulation with age, oxidative phosphorylation was investigated by measuring P/O ratio in mitochondria isolated by differential centrifugation. There was no difference in P/O ratio (1.8 to 2.8) in mitochondria from kidneys of fetal, newborn, or adult rabbits indicating that differences in PAH accumulation could not be explained by a deficiency in this system.

* By Invitation

154. The Intracellular Cation Composition of Skeletal Muscle in Congenital Heart Disease

DONOUGH O'BREN,* Colorado
Introduced by HENRY K. SILVER.

This study was undertaken to determine the effects of cardiac insufficiency, anoxia and/or hypothermia on the intracellular electrolyte pattern in congenital heart disease, and to correlate the findings with the clinical course during and after surgery. A series of skeletal muscle samples of approximately 200 mg. from normal children and from those with congenital heart disease were analyzed for sodium, potassium, magnesium, chloride, nitrogen, and phosphorus content.

In calculating the intracellular cation composition, the assumption was made that the chloride was wholly extracellular. On this basis the variations in intracellular ionic composition in patients with congenital heart disease appeared to fall into three groups as shown in the table below.

Group I. Moderate replacement of intracellular potassium by sodium.

Group II. The apparent intrusion of substantial amounts of chloride as well as sodium into the cell.

Group III. Marked diminution in the total intracellular cation concentration.

The significance of these findings will be elaborated and discussed.

	Cardiac Failure	Intracellular Cations, mEq/L. Potassium	Sodium	Magnesium
Normal Children	none	172-188	2-13	34-46
Children with congenital heart disease				
Group I	Minimal or none	103-153	6-93	33-46
Group II	none	69	118	40
	none	124	65	40
Group III	++	95	31	31
	++	118	0	20
	++	86	74	9

The significance of these findings will be elaborated and discussed.

155. In Vitro Studies of the Effect of Sulfonamides on Bilirubin

GERARD B. ODELL,* Johns Hopkins
Introduced by ROBERT E. COOKE.

Previous reports have shown that there is a correlation of kernicterus with hyperbilirubinemia

and that kernicterus has occurred more frequently at lower plasma bilirubin concentrations in prematures than in full-term infants. The occurrence of kernicterus is more frequently observed in newborn babies and animals treated with certain sulfonamides than in comparable controls. However, little is known of the movement of bilirubin from plasma into cells and how this movement is enhanced by sulfonamides.

The present studies report the effect of the sulfonamides, sulfisoxazolediethanolamine and sodium sulfadiazine on serum bilirubin from two aspects: (*a*) ultrafiltration and dialysis of serum bilirubin, and (*b*) spectrophotometric absorption patterns.

Serums containing high indirect bilirubin concentrations (10 mg. % to 28 mg. %) were ultrafiltered in a Lavietes unit. Other samples of these same sera, to which sulfonamide had been added to give concentrations of 5 mg. % to 20 mg. %, were simultaneously ultrafiltered in another Lavietes unit. The ultrafiltrates from the serums alone were colorless and showed no evidence of bilirubin by spectrophotometric analysis or by the van den Bergh reaction. The ultrafiltrates of the sera treated with sulfonamides were colored yellow and showed an absorption peak at 430 mμ characteristic for bilirubin in nonprotein-containing solutions. Addition of the diazo reagent further showed disappearance of this peak and a new peak appeared at 540-580 mμ, characteristic of bilirubin coupled with diazotized sulfanilic acid. The concentrations of bilirubin in the ultrafiltrates averaged 0.5 mg. % This concentration is greater than concentrations one can get of bilirubin in buffer solutions containing bilirubin at pH 7 if no protein is present, and it suggests that a water-soluble complex may have formed.

Dialysis studies in progress have thus far demonstrated that the sulfonamides enhance the dialysis of bilirubin across cellophane membranes.

Spectrophotometric studies of serum with varying concentrations of bilirubin reveal that the absorption maxima at 460 mμ is depressed by sulfonamides. The amount of depression varies directly with the concentration of sulfonamide present, and behaves like substances which interfere with organic anion binding to proteins. These studies indicate that sulfonamide displaces bilirubin from its protein-binding sites on serum proteins, allowing more rapid diffusion to other body compartments.

156. Thyroidal Secretion and Degradation Rates in Children

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Introduced by JACK METCOFF.

In a previous study, using radioiodine, of thyroid function in children it was found that the means and standard deviations of 24 hour thyroidal uptake values did not differ from those of adults but the radioactive protein-bound iodine^{131} (PBI^{131}) levels were elevated up to five years of age and probably longer. The data were interpreted as consistent with increased thyroidal secretion, although a smaller thyroidal organic iodine pool with less dilution of concentrated radioiodine could also result in greater tagging of secreted hormone.

To elucidate the mechanism of the elevated PBI^{131} levels, secretion rates of thyroidal radioiodine (with tapazole block) were performed on five children without thyroid disease, ages three to eight, and on four young adults. The mean thyroidal secretion rate was 14.2% per day in the children and 3.8% in young adults. In the children the peripheral degradation rate, the thyroidal and extrathyroidal organic iodine pool content, and the quantity of organic iodine secreted and degraded per day were calculated from the thyroidal secretion rate, the slope of PBI^{131} rise, and the serum protein-bound iodine determination. The volume of distribution of thyroxin was assumed to be 15% of body weight. In children the peripheral degradation rate was increased five fold, while the quantity of thyroidal organic iodine was diminished as compared to adult values. Children secreted and degraded an average of 93.2μg. of hormonal iodine per day (213.3μg/1.73 square meter of surface area) compared to reported values of 50-70μg. per day for adults.

These data indicate a relative physiologic thyroidal hyperactivity in children as compared to adults.

157. Extrarenal Changes Occurring in Nephrosis During Steroid Diuresis

WILLIAM J. OLIVER* and CHARLES SLOAN,* Michigan

Introduced by BRUCE D. GRAHAM.

Alterations in serum oncotic pressure and in plasma volume have always been considered to play a critical role in the pathogenesis of the severe edema of active nephrosis. However, the contribution of these same factors and also other "extrarenal" events to the reversal of the edematous state have received less attention than the demonstrable improvement of renal function which occurs at the time of diuresis.

* By Invitation

The present study has been directed towards observations of the possible role of these "extrarenal" factors during the transition from the edematous to the nonedematous phase of nephrosis.

Since equations for the estimation of serum oncotic pressures have not been definitely established for the low ranges and abnormal distributions of serum proteins encountered in nephrosis, direct measurements of such pressures are essential for determining sequential changes. In the present study a micromethod (modified after that of Holm-Jensen) utilizing 0.35 ml. of serum has been employed. This method has been calibrated in our laboratory by the determination of the molecular weight of purified human serum albumin, and it has given satisfactory results.

In children with nephrosis, serial determinations of serum oncotic pressures have been obtained prior to, during and subsequent to the diuresis induced by steroid therapy. Simultaneous measurements of total serum protein concentration, serum electrophoretic pattern, serum lipid and cholesterol, and serum electrolytes have been made. In addition, in the majority of subjects, plasma volumes, and the trend of aldosterone activity have been determined. Urinary Na/K rations have been used as an index of the latter.

Preliminary studies indicate that diuresis occurs when serum oncotic pressure and serum albumin concentration have increased in magnitude only minimally above the levels observed during severe edema, and that return to normal levels occurs only several weeks subsequent to complete remission. A similarly delayed return to normal has been noted for the remaining protein fractions. Concurrent measurements of the serum cholesterol concentrations at the onset of diuresis likewise demonstrate no significant changes from the prediuresis values. No definite trends have been established for the remaining factors under investigation.

Although there are a number of possible interpretations, the observations suggest to us that either the diuresis induced by steroid therapy in nephrosis is dependent almost wholly upon alterations of renal function, or that extremely small increases in oncotic pressure may result in large movements of water (and electrolytes) from the interstitial compartment to the intravascular compartment, with the resultant renal excretion of water and electrolytes as a secondary phenomenon.

158. Cinefluorography with Sound: A Technique for the Evaluation of Motor Activity of the Palate and Speech

RICHARD W. OLMSTED,* and JOHN A. KIRKPATRICK JR.,* Temple
Introduced by JAMES B. AREY.

Cinefluorography utilizing an image intensifier and the simultaneous optical recording of sound offers a method of visualization of the movements of the palate during speech. This technique affords a means of investigation of the movements of the palate, tongue, and soft tissues of the pharynx, and their relation to velopharyngeal closure and voice production. In children with cleft of the palate, information obtained from these studies aids in the decision as to surgical or prosthetic closure of the palate and in the evaluation of the adequacy of closure. Furthermore, it provides a means of continuous evaluation of the progress of the child during long term speech therapy.

In addition to children with clefts of the palate, those with palatal paralysis and foreshortening of the palate without clefts are being investigated. Children without speech or palatal defects are also under study to determine the normal pattern of motor activity of the palate and associated structures.

Representative sound movies will be shown to illustrate the application of this technique.

159. A Study of the Effect of High Oxygen Concentrations on the Opossum Pouch Young

ARTHUR H. PARMELEE JR.; MILO B. BROOKS,* and ROBERT S. STONE,* U. C. L. A. School of Medicine, Los Angeles

The young of the Virginia opossum, Didelphis Virginiana, are born 12 to 13 days after conception. They migrate immediately into the pouch of this marsupial animal where they remain for 75 to 80 days. At birth the general development of the opossum young is roughly comparable to that of the human fetus of three months. Therefore, the opossum pouch young would appear to be useful animals for studies in the management of the prematurely born.

One of the recent serious complications of the care of premature infants has been that of oxygen poisoning. This is manifest as retrolental fibroplasia. High oxygen concentrations seem to have their greatest effect on newly developing capillaries as demonstrated by Patz and Ashton. It is feasible that capillary damage may occur in other areas of the body, particularly in the brain. Hepner has been able to produce neurological disturbances and histological evidence of perivascular hemorrhages in the brain of newborn mice when the mothers were subjected to high oxygen concentrations just before delivery.

In our study two opossum mothers, Subject A and Subject B, with litters of 11 and 9 young respectively, were exposed to high oxygen concentrations. Subject A remained continuously in an atmosphere of 60% oxygen from the 2nd to the 52nd day after the birth of her young, and Subject B, from the 2nd to the 21st day. Control animals A and B were kept in similar circumstances as Subjects A and B, but in normal concentrations

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of oxygen. Control A had a litter of 12 young and Control B, 7 young. In each instance the control animals were born on the same day as the subjects.

The oxygen concentrations were checked with the Beckman oxygen analyzer. Previous reports by Patz have indicated that oxygen concentrations of 70% or higher are lethal to the mother opossums within four days. Therefore, oxygen concentrations were maintained between 50% and 60%.

Oxygen and carbon dioxide concentrations were not checked in the pouches of these experimental animals, but were studied later in another experimental animal with six pouch young 50 days of age. This animal was kept in oxygen of 50% to 60% concentration for 24 hours. Gas samples from the pouch were taken at varying intervals and analyzed for CO_2 and oxygen by the Scholander method. Pouch oxygen concentrations ranged from 41% to 55% and carbon dioxide concentrations from 1.06% to 3.63%.

Pouch young were removed from Subject A and Control A at ages 11, 29, 30, and 50 days and from Subject and Control B at ages 2, 4, 11, 17, and 21 days. No differences were noted in their growth rates. Microscopic studies revealed no pathology in the eyes, brains, or lungs of either the subject or control animals. Serial sections of the 11-day-old animals revealed no obvious pathology in any tissues. Illustrations of the pouch young and of the microscopic sections will be presented.

It is possible that the oxygen concentration used was not great enough to be noxious, that the increased carbon dioxide concentration offered some protection, or that some metabolic factors in the opossum pouch young are protective against the noxious effects of increased oxygen concentrations.

160. An Immunologic Assay for Minute Amounts of Human Pituitary Growth Hormone

CHARLES H. READ and DANIEL B. STONE,* Iowa

A simple and sensitive immunologic method has been developed for the assay of human pituitary growth hormone in serum. Boyden (J. Exper. Med. 93:107, 1951) showed that treatment of sheep erythrocytes with tannic acid rendered them capable of adsorbing protein molecules from solution; red cells thus prepared were agglutinated by homologous antiprotein serum. In the present study, it has been found that tannic acid treated sheep erythrocytes adsorb human growth hormone (prepared by Dr. Raben) and that they agglutinate in the presence of rabbit antihuman growth hormone serum.

The procedure for the assay of human pituitary growth hormone (PGH) comprises three stages. In the first stage, sheep erythrocytes are treated with dilute solutions of tannic acid; the cells are then washed and exposed to a solution of PGH. After further washing, they are added to serial dilutions of rabbit anti-PGH serum. Hemagglutination occurs in the higher concentrations of the antiserum. The highest titer of anti-PGH serum which agglutinates the antigen-treated erythrocytes is thus determined.

In the second stage, decreasing known concentrations of PGH are added to a predetermined dilution of rabbit antiserum. The PGH inhibits the activity of homologous antiserum. PGH-treated sheep erythrocytes are now agglutinated only in the tubes containing lesser quantities of PGH, since the known added amount of PGH is here insufficient to inactivate all of the antibody. The minimal amount of growth hormone with which the rabbit antiserum reacts is thus found.

In the third stage, serial dilutions of human serum are substituted for the known PGH dilutions used in the second stage. That dilution of serum which just inhibits hemagglutination is thus established; this dilution contains the same amount of PGH which similarly inhibits hemagglutination in the second stage. The concentration of hormone in the serum is then calculated.

Many factors influencing the test have been studied. These include the effect of pH on the stability of the tanned erythrocytes after exposure to PGH, the dilution and type of animal serum used as a diluent, the concentration of the erythrocytes, and the amount of PGH exposed to the tanned cells.

The specificity of the test has been analyzed. No circulating PGH has been found in the sera of the two hypophysectomized patients tested. Tanned sheep erythrocytes exposed to either insulin, TSH, or beef growth hormone are not agglutinated by the rabbit anti-PGH serum. Further, these protein hormones fail to inhibit the antiserum in hemagglutination inhibition tests. Other fractions extracted from the same human pituitaries from which the PGH was prepared, namely "supernatant," pH 8.5 precipitate, and corticotrophin have been examined. Hemagglutination of tanned erythrocytes exposed to these fractions occurs only with the "supernatant." This "supernatant" also inhibits rabbit anti-PGH serum in hemagglutination inhibition tests; pH 8.5 precipitate and corticotrophin have little inhibiting ability. Human PGH, prepared by Dr. Li, has been tested and shown to have immunologic characteristics which are identical to the PGH used in these experiments.

* By Invitation

161. Treatment of Idiopathic Female Sex Precocity with Synthetic Progestational Compounds

ROBERT RICHIE* and JOHN CRAWFORD, Harvard

Though idiopathic central precocious puberty in the female carries a benign prognosis with respect to longevity and fertility, the desirability of finding satisfactory therapy is pointed up by the harmful effects of the condition on the patient's developing personality, the threat it frequently represents to the family, the occurrence in adult life of short stature, and the danger of sexual violation. This preliminary report summarizes experience to date in treating one patient from 18 to 36 months of age with 19-nor-17-ethinyl testosterone and another patient from five and one-half to seven years of age with ethinyl testosterone. Doses of 1.25 mg. per day of the 19-nor-17-ethinyl testosterone stopped the preexisting regular menses and caused regression in breast size without androgenic side effects, while 10 mg. per day were adrogenic and 0.2 mg. per day allowed recurrence of menses. In the second patient doses of 50 and 75 mg. per day of ethinyl testosterone have been without undesirable side effects.

In both patients there was a striking diminution in signs of estrogen effect as judged by changes in breast size, vaginal smears and inhibition of menstrual bleeding. In addition, the accelerated linear growth rates were slowed to normal or subnormal levels. In the first patient skeletal maturation has advanced in an unabated fashion while in the second it has apparently been slowed to a rate commensurate with that of linear growth. In both girls, signs of estrogen effect have reappeared rapidly on temporary withdrawal of medication.

Urinary gonadotropin excretion has persisted at adolescent levels and in the first patient urinary estrogen excretion remains elevated while the signs of estrogen effect are diminished. One male patient with idiopathic precocity treated with both steroids and another treated only with the 19-nor derivative showed no favorable response.

It appears that these agents act to antagonize estrogen rather than to supress pituitary gonadotropin or inhibit estrogen elaboration at the ovary. The slowing of linear growth rate seems best explained by assuming that the low titers of endogenous estrogen characteristic of this syndrome are in part responsible both for the rapid linear growth and premature closing of the epiphyses.

162. An Investigation of Maternal Concerns

WILLIAM O. ROBERTSON,* Ross Laboratories, Columbus, Ohio

Introduced by L. J. FILER JR.

During recent years, pediatrics has directed increasing attention toward comprehensive child health supervision. An important facet of such a program is the pediatrician's function in advising, instructing, and reassuring mothers about those concerns commonly associated with child rearing. A review of the pediatric literature, however, reveals no extensive data pertaining to the relative incidence of different maternal concerns or the influence on incidence of such factors as maternal age, maternal parity, infant's sex, or geographical location of the family. In an attempt to gather this data, a survey of mothers was undertaken.

A simple questionnaire was devised, pretested, and then forwarded by mail to a nationwide sample of 2000 mothers of young babies. Of the 1875 questionnaires delivered, 1132 (60.3%) were completed and returned. Based on several variables, comparison of the distribution of this sample with government figures revealed a random distribution had been achieved except, as was anticipated, with regard to the respondents' level of educational attainment and to urban-rural distribution.

Manifestations of possible gastrointestinal dysfunction, for example, "gas," hiccups, "spitting up," skin disorders, and sleep disturbances, were the most frequently noted sources of concern. Concern about possible anatomic deviations from normal was considerably less frequent.

Increase in maternal age or level of education had little influence in decreasing the incidence of the respondents' concerns. In contrast, increase in multiparity had considerable effect. However, by the mother's definition, apparently the incidence of worry about "colic" remained stable in spite of an increase in multiparity. Neither variables within the infant, such as birth weight or sex, nor varying geographical location or urban-rural distribution of the family, in general, appeared to influence as significantly the incidence of maternal concerns as did increase in parity of the mother.

Other correlations of possible significance will be considered. It is suggested that the technique of mail questionnaire survey offers a reliable method for collecting objective data from a widespread population in a brief span of time, which data, when properly interpreted, may be applied with advantage in approaching problems of comprehensive child health supervision.

163. Clinical Recognition of the Transitional Pulmonary Circulation of Newborns: A Pilot Study Using Auscultation and Electrocardiography

RICHARD D. ROWE, Toronto

Previous hemodynamic studies have confirmed that a phase of pulmonary circulation transitional between fetal and adult forms occurs in normal newborn infants as well as in a variety of animals. During this period pulmonary hypertension and a significant volume left-to-right shunt through a

* By Invitation

patent ductus arteriosus exists. Data for humans have suggested that this transition does not normally last longer than two weeks. The present study was taken to determine whether clinical correlation between the hemodynamic data and auscultatory and electrocardiographic phenomena existed during this particular period.

Auscultation was performed in 15 mongoloid infants at varying ages from 56 hours to 9 months and in five normal healthy newborn infants during the first four hours of life. Electrocardiograms from 12 mongoloid infants subjected to right heart catheterization and 21 healthy newborn infants were analysed.

Infants over the age of nine days uniformly had normal heart sounds with a moderate degree of splitting of the second heart sound in the pulmonary area and no cardiac murmurs. Their electrocardiograms all had negative direction of the T wave in Lead V_1 of the electrocardiogram. Infants under the age of 12 days had variable alteration in the second heart sound in the pulmonary area depending upon the level of the pulmonary arterial pressure. Healthy newborn infants in the first four hours had accentuated pulmonary valve closure with pulmonary ejection clicks in addition. The T wave was positive in V_1 for a varying period after the first hour of life. Murmurs were not audible during the first four hours of life but were present in a proportion of the infants after that age until 12 days.

These preliminary studies suggest that marked pulmonary hypertension of the high vascular resistance type is present in the first few hours of life and that pulmonary hypertension may be recognized on clinical grounds at this stage. Electrocardiographic data suggest that some time between 24 and 96 hours, the pulmonary arterial pressure falls to a mature level in the majority of normal infants and that during this time a murmur should be audible. This information has been of considerable practical help in assessing the state of pulmonary circulation of newborns particularly those with abnormal pulmonary ventilation and congenital cardiac defects.

164. A Study Concerning the Basic Metabolic Defect in Wilson's Disease

ANDREW SASS-KORTSAK; ROBERT J. SLATER; DOUGLAS G. GEIGER,* and MORRIS CHERNIAK,*
Toronto

In the course of a study of copper metabolism in Wilson's disease, two brothers suffering from the hepatic form of this conditon were found to have normal levels of ceruloplasmin in the serum by several methods of determination. The diagnosis of these two patients was based on the finding of an increased urinary copper excretion, aminoaciduria, and the presence of Kayser-Fleischer rings. In addition markedly increased amounts of copper were found in the liver, kidney, and brain of the one patient so studied.

The finding of normal ceruloplasmin levels in Wilson's disease is unusual in view of the widely quoted theory that a defect in the synthesis of ceruloplasmin leading to low blood levels of this protein is the basic abnormality in this condition. Since a defect in the incorporation of copper into ceruloplasmin has been well substantiated in patients suffering from Wilson's disease and having low ceruloplasmin levels, it seemed of interest to carry out such a study on one of our patients.

A single oral dose of Cu^{64} representing 1 mC. activity was given orally. Total blood, plasma, and albumin-bound activities were measured in serial samples collected during the next 60 hours. Quantitative collections of urine and stool were also assayed for radioactivity. A similar experiment was performed on another case of Wilson's disease, a 12-year-old girl showing all the typical laboratory and clinical characteristics of the condition, including consistently low serum ceruloplasmin levels.

Both these patients, one with normal and the other with extremely low serum ceruloplasmin level, behaved in a similar fashion. The findings were typical of Wilson's disease in that evidence could not be obtained of incorporation of copper into ceruloplasmin during the study period. In addition to this both eliminated a far larger percentage of the administered copper in the urine than the normal individuals tested similarly by Jensen and Kamin.

The occurrence of normal ceruloplasmin levels in patients who otherwise display the usual characteristics of Wilson's disease suggests that the actual deficiency of this protein in the blood is not the essential defect.

The coexistence of a normal ceruloplasmin level in the serum with a defect in the incorporation of copper into this protein is extremely difficult to interpret in the light of present knowledge. As a working hypothesis it is suggested that an exchange process between ceruloplasmin-bound and relatively free copper may be disturbed. The existence of such an exchange process was suggested by the in vitro experiments of Sheinberg, but since the physiological occurrence and significance of this is not yet proven such interpretation has to remain in the realm of theory.

165. Ebstein's Anomaly of the Tricuspid Valve: A Clinical Analysis of Twenty-Four Cases

GEROLD L. SCHIEBLER*; PAUL ADAMS JR.; RAY C. ANDERSON; KURT AMPLATZ,* and RICHARD G. LESTER,* Minnesota

Among the anomalies of the tricuspid valve, the downward displacement of any portion has been called Ebstein's malformation.

* By Invitation

During the past decade, we have seen 24 cases with this malformation, eight of which have had postmortem examination. This constitutes the largest series ever assembled. The rapidity with which new cases are being reported indicates that this anomaly is not uncommon.

Neonatal symptoms were a prominent feature. Where recorded, most cases had cyanosis and/or a murmur. Significantly, in all our cases the cyanosis decreased or disappeared entirely with time.

Four of our cases now living have no symptoms. In the others, fatigue, cyanosis, bout of tachycardia, central nervous system symptoms, and chest pain were prominent features. Terminally, heart failure and thromboembolic phenomena were also noted. Cyanosis showed no relation to exercise tolerance.

These individuals were usually of normal weight and height. There was no correlation between degree of cyanosis and physical development.

The examination of the heart invariably showed cardiomegaly, the pulmonic second sound equal or greater than the aortic second sound, variable systolic and/or diastolic murmurs. However, in the majority of cases, one could note the "triple" or "quadruple" rhythm. This alone may suggest the diagnosis.

Phonocardiograms showed a split first sound, a second sound, a low frequency third heart sound, and on occasion a presystolic sound or murmur.

Roentgen examinations showed that the heart could be normal size or massively enlarged. However, they could be roughly divided into two basic groups; (1) the markedly enlarged "box-shaped" heart caused by right atrial and right ventricular outflow tract dilatation, and (2) a moderately enlarged heart with a sloping left cardiac contour which resembled left ventricular hypertrophy. In spite of massive cardiac enlargement, no case showed left atrial enlargement but did show the usual criteria or pulmonary congestive changes. No case showed increased pulmonary markings.

The electrocardiograms could also be divided into two groups: (1) those having the Wolff-Parkinson-White pattern, and (2) those having incomplete or complete right bundle branch block. In six of our cases, the W-P-W pattern was noted, and this pattern was also seen on the original case reported from this medical center by Yater and Shapiro. This unusual combination in our series is about as large as all other cases previously reported in the literature. We feel that the clinician seeing a patient with the W-P-W configuration, cyanosis, and normal or decreased markings must consider this anomaly. The other cases showed incomplete or widely splintered complete right bundle branch block plus, on occasion, a prolonged P-R interval, "P pulmonale," right axis deviation, and low voltage in the right precordial leads. In none of our cases was the R wave in lead V_1 over 8 mm. in height.

Twenty-two catheterizations have been performed without any morbidity or mortality. These usually showed the tricuspid valve to be displaced to the left in the A-P view, a large right atrium, elevated right atrial pressure, and normal or slightly elevated right ventricular and pulmonary artery pressure with little or no gradient between them.

Operation has been attempted on three cases utilizing total cardiopulmonary bypass with the pump oxygenator. All were terminal cases. One died during the induction of anesthesia, the other two died within hours after the completion of the operation. The operation consisted of plication of the "atrialized" right ventricle bringing the leaflets up to the true annulus fibrosis, plus closure of the foramen ovale.

Autopsy specimens showed various tricuspid valvular malformations, usually a functional anterior leaflet, a "sessile" septal leaflet, and a rudimentary posterior leaflet.

The pathophysiology appeared to range from tricuspid stenosis to marked tricuspid insufficiency or probable degrees of each.

The lesion is compatible with long life or it may cause neonatal death, the average life span being between twenty to thirty years.

166. Studies of Serum Ceruloplasmin During Early Infancy

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Albert Einstein College of Medicine
Introduced by HENRY L. BARNETT.

Approximately 95% of the serum copper is normally bound to a blue protein, ceruloplasmin. This is an α-globulin with a molecular weight of approximately 150,000 and a copper content which corresponds to eight atoms of copper per molecule. This concentration of ceruloplasmin in the serum of normal adults ranges from 15 to 30 mg. per 100 ml. Concentrations above normal are seen in pregnancy and a variety of disease states, while concentrations below normal are seen in patients with the nephrotic syndrome, and hepatolenticular degeneration (Wilson's disease).

We have measured serum ceruloplasmin concentration in 20 pairs of mothers and infants at delivery. The serum ceruloplasmin concentrations were then determined serially in most of these infants during the first four months of life.

The results obtained indicate that ceruloplasmin concentrations are low at birth, and increase during the first few weeks of life. Considerable variation was noted in the concentration of ceruloplasmin in cord blood, and in the rate at which it rose toward normal.

* By Invitation

These results will be presented, and discussed in respect to (1) the relation of the concentration of ceruloplasmin in the serum of cord blood to that in maternal blood; (2) the relation of the cord blood ceruloplasmin concentration to the infant's sex, birth weight, and gestational age; (3) the age at which ceruloplasmin levels approach normal adult values, and (4) the relation of infection during early infancy to the rate of rise in serum ceruloplasmin concentration.

167. Glutathione Metabolism in Cord Blood and in the Newborn Infant

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Introduced by RUTH T. GROSS.

A hereditary abnormality of the red cells was described in Negroes sensitive to primaquine.

A low reduced glutathione (GSH), a GSH instability as well as a low activity of glucose-6-P dehydrogenase was demonstrated.

A similar abnormality was described in non-Ashkenasic Jews (Mediterranean) but not Ashkenasic (East European) Jews.

To study the earliest appearance of this abnormality of the GSH level, its stability as well as glucose-6-phosphate dehydrogenase levels were determined on cord blood and newborn infants' blood on a heterogenous Jewish population in Israel. One-hundred-seventy-two samples of cord blood from non-Ashkenasic subjects and 52 from Ashkenasic subjects were tested. The cord blood showed a higher GSH level than adult blood, 82.2±13.4 mg/100 ml., control 50.4±13.5 mg/100 ml.

The hereditary abnormality could be detected by using the GSH stability test of Beutler, or glucose-6-phosphate dehydrogenase activity determination. When newborn infants were examined at the age of 30-75 hours a GSH instability was found. This finding was described by Zinkham and Childs. However, this GSH instability was non-hereditary. It appears in Ashkenasic and non-Ashkenasic Jews.

Glucose or inosine normalized the instability and glucose-6-phosphate dehydrogenase levels were found to be high.

The incorporation of glycine 1-C^{14} into red cell GSH of those newborns was normal while the incorporation in the hereditary defective cells was low. This GSH instability disappeared on the eighth day.

The possible mechanism of the nonhereditary GSH instability in newborns will be discussed.

168. The Pathogenesis of Proteinuria

R. J. SLATER; D. W. GEIGER,* and C. P. RANCE,* Toronto

The presence of protein in the urine is the most common clinical manifestation of renal dysfunction. "Urinary protein" varies widely in quantitative excretion as well as qualitative characteristics. In order to understand better the manner by which certain diseases alter kidney function and induce these variations, we have continued our studies of the clearances of specific plasma proteins of differing weights and dimensions.

In more than 130 tests on 28 children with postural proteinuria, nephrosis or acute or chronic nephritis, clearances of albumin (alb., M. W. 68,000) iron-binding globulin (fe.-glob., M. W. 90,000) and γ-globulin (γ-glob., M. W. 150,000) have been estimated by means of quantitative precipitin procedures. In certain patients these have been compared with the clearances of creatinine, inulin, and para-aminohippurate. When the clearance of one of the globulins is expressed as a percentage of the clearance of albumin $\left(\frac{C_{\gamma\text{-glob.}}}{C_{alb.}}\ \%\right)$ serial changes in the renal behavior with respect to these proteins will be reflected independently of fluctuations in total protein clearance.

During postural proteinuria a consistently similar pattern of protein clearance is found in different children, and in the same child during marked variation in proteinuria. In this group there occurs a high value for the ratio $\frac{C_{\gamma\text{-glob.}}}{C_{alb.}}\%$ (50-80) and also $\frac{C_{fe\text{-glob.}}}{C_{alb.}}\%$ (90-100). The electrophoretic pattern of this type of urinary protein is very similar to that of the serum.

In children with the nephrotic syndrome, serial observations during relapse reveal little daily change in the total and individual protein clearances. The increased permeability seems to be in a fixed state with usually a low ratio $\frac{C_{\gamma\text{-glob.}}}{C_{alb.}}\%$ (0.5-1.0) but a high ratio $\frac{C_{fe\text{-glob.}}}{C_{alb.}}\%$ (80-100).

During clinical improvement in nephrosis from relapse to remission, serial analyses revealed that decreasing total protein clearances occur in the children. However, the individual protein clearance ratios behave differently, $\frac{C_{\gamma\text{-glob.}}}{C_{alb.}}\%$, increases from 1 to 15-18, while $\frac{C_{fe\text{-glob.}}}{C_{alb.}}\%$ remains unchanged (80-100). Thus a distinct alteration in relative permeability to certain proteins of different size occurs during changes in total protein permeability in nephrosis.

Upon infusion of human serum albumin into patients with nephrosis, an acute increase in total protein clearance follows. However, no change is found in the values $\frac{C_{\gamma\text{-glob.}}}{C_{alb.}}\%$ or $\frac{C_{fe\text{-glob.}}}{C_{alb.}}\%$ during these periods. It is clear that with the increased excretion of albumin, there occurs, in a proportional manner, an increased excretion of other proteins.

On the assumption that glomerular filtration and tubular reabsorption control renal permeability to protein, evaluation of these variations in relative

* By Invitation

protein clearances permits the following hypotheses: (1) increases in glomerular permeability, mainly to smaller molecular weight plasma proteins, determine the relative composition of urinary protein; (2) any tubular reabsorption of these proteins appears to be competitive, being proportional and nonpreferential in nature.

Further study is required to assess the extent and selectivity of tubular reabsorption of filtered plasma proteins.

169. Poliovirus Antibody in Cerebrospinal Fluid

ALEX J. STEIGMAN; MURRAY M. LIPTON,* and FE C. DIZON,* Louisville

In 1949, Morgan reported antibody to Type 2 poliovirus in the spinal fluid and spinal grey matter of monkeys previously infected with such virus; she found on the other hand that monkeys vaccinated with formolinized virus developed good serum antibody titers without demonstrable antibody in their spinal fluids. It was considered that the specific antibody in the infected monkey's spinal fluid was fabricated locally, perhaps from inflammatory cells in the CNS, and that antibody in the serum of vaccinated animals did not traverse into the CSF. Employing Type 1 poliovirus strains of recent human origin in tests performed in monkeys, Sabin and Steigman were unable to disclose polio antibody in the spinal grey matter of convalescent monkeys.

A group of children with clinical poliomyelitis due to Type 1 poliovirus was recently studied. Simultaneous tests were made of the neutralizing capacity of each patient's serum and CSF against 100 tissue-culture doses of the three serotypes of poliovirus. Serial CSF samples in convalescence when the globulin content is elevated were not available. Nevertheless, specific antibody to Type 1 poliovirus was demonstrable in some patients, the highest observed titer being 1:32. Some of the children had serum antibody against Type 2 and Type 3 poliovirus, presumably due to "inapparent" previous infection as none had a history of prior poliomyelitis. Thus, one of these Type 1 poliovirus infected children simultaneously revealed a serum titer of 1:512 and a CSF titer of 1:64 to Type 2 virus. Does this represent active transport of specific antibody molecules from the serum to the CSF in some arbitrary ratio or does local inflammation of the CNS occur regularly with "inapparent" poliovirus infection of the alimentary tract?

Whether Morgan's observations in mouse tests that adequately vaccinated monkeys fail to show polio antibody in their CSF applies to children is not known. Suitable test circumstances would require that such vaccinated children have had no previous immunological experience with poliovirus. Antibody tests of spinal fluids of such children could clarify whether "selective seepage" of antibody molecules occurs from the serum or whether the local CNS tissues must be antigen-sensitized for antibody to appear.

* By Invitation

170. The Effects of Large Doses of Salicylate on the Metabolism of Cortisol in Human Subjects

ROBERT STEMPFEL*; JAMES B. SIDBURY, and CLAUDE J. MIGEON, Johns Hopkins

The present study is an attempt to elucidate the metabolism of cortisol in human subjects receiving acetylsalicylic acid in therapeutic dosage.

The mode by which salicylates exert their antirheumatic effects is unknown. It has been postulated by many that the phenomenon may be at least partly dependent upon a direct or indirect stimulatory effect of the drug upon the pituitary-adrenal axis. Since no consistent elevation of plasma 17-OH-CS has been observed in normal subjects receiving therapeutic doses of salicylate, it has been suggested that there might be an increased production of corticoids accompanied by a similarly increased rate of their removal from the circulation.

In order to clarify further the metabolism of cortisol in subjects receiving large amounts of salicylate, 4-C^{14}-cortisol was administered intravenously to normal individuals given 6 to 8 gm. of acetysalicylic acid daily. The half-life of both the free and glucuronide radioactivity in the plasma was found to be within the normal range, and the excretion of total radioactivity in the urine was entirely consistent with these findings. However, using the usual techniques of hydrolysis with β-glucuronidase, the urinary glucuronide fraction was found to be markedly diminished. It is probable that under these circumstances the hydrolysis of urinary corticosteroids is competitively inhibited by the large amounts of salicylate glucuronide present. When very large amounts of the enzyme were employed and/or the incubation period was sufficiently prolonged, complete hydrolysis of the corticoid glucuronides could be obtained and the rates of excretion were within the range seen in normal subjects who had not received salicylate. The inhibitory effects of salicylate glucuronide may explain the low values of urinary 17-OH-CS reported by others. Similar studies, using nonradioactive cortisol were performed and tend to confirm these findings.

It is concluded that acetylsalicylic acid, in the dosage employed, does not cause an increase in the rate of utilization of cortisol.

171. Angiocardiographic Diagnosis of Aberrant Left Coronary Artery from Pulmonary Artery

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Introduced by BRUCE D. GRAHAM.

The diagnosis of an aberrant left coronary artery in infancy may be confused with a number of conditions formerly included under the general diagnosis of idiopathic hypertrophy of the heart. Although the presence of an electrocardiographic infarction pattern is highly suggestive of this lesion, the same pattern has been seen in other conditions at this age. The accurate diagnosis of the anomalous left coronary artery is important because it is the one condition for which there is hope of corrective surgery. Furthermore, if the surgical transplantation of the aberrant coronary artery into the aorta is to be of maximal benefit, it would be desirable for the operation to be undertaken before myocardial infarction had occurred.

Through the use of angiocardiography it has been possible to demonstrate the origin of the left coronary artery from the pulmonary artery. Cases demonstrating the use of this technique will be shown.

172. An Epidemiologic Study of the Nephrotic Syndrome

GUNNAR B. STICKLER,* Mayo Clinic
Introduced by ROBERT A. GOOD.

Epidemiologic methods were used for a survey of nephrotic children admitted to all hospitals with a pediatric department in the Greater Buffalo area between 1940 and 1957. It was assumed that all children with classical nephrotic syndrome were first hospitalized in a hospital nearby their residence. By using Barnett's criteria for the diagnosis of nephrotic syndrome, 72 patients suffering from this disease were found. The incidence was found to be 2.1 per 100,000 children (0 through 9 years old) per year. A higher incidence (4.8) was found among the nonwhite population. No indication of seasonal variations of the time of onset was found. No geographic accumulation of cases was demonstrated nor was any correlation between incidence of nephrosis and certain socioeconomic groups found. The sex ratio of nephrotic children living inside the city limits of Buffalo was very significantly different from that of nephrotic children living outside the city limits. The ratio, male: female, was within city limits 31:6, outside city limits 16:19. No explanation for this difference was found.

* By Invitation

173. Hepatic Fibrosis in Children with Acute Leukemia

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Chemotherapeutic agents produce temporary clinical and bone marrow remissions and prolong life in children with acute leukemia. These patients are usually maintained on antimetabolites, such as amethopterin or 6-mercaptopurine during the major portion of their illness (one to two years), and it is of interest to determine whether any chronic tissue injury has resulted from this prolonged treatment. Hepatic fibrosis in three children with acute leukemia treated with aminopterin has been reported previously (Colsky et al., A. M. A. Arch. Path. 59:198, 1955). The present study was designed to correlate the possible role of specific chemotherapeutic agents and other factors in the production of hepatic fibrosis. Three-hundred-two autopsies performed on children with neoplastic disease at Memorial Hospital during the period 1940-1957 were reviewed, and the liver pathology was correlated with the clinical histories. Data on each patient included the age, sex, relative nutritional status, extent of the disease involvement at the onset, the type of chemotherapeutic agents used and the method and duration of their administration, the response of the disease to therapy, the frequency of blood transfusions, clinical evidence of hepatitis, and the survival time both from the onset of the disease and from the beginning of chemotherapy. Two-hundred-sixty-five were diagnosed as acute leukemia (six received no form of chemotherapy), and 38 had other forms of neoplasms and were treated with chemotherapeutic agents. In addition liver sections on 18 children who died accidentally or after a short term illness were obtained from a medical examiner. The liver sections were stained with Hematoxylin and Eosin, Masson's, reticulum, and elastic tissue stains.

According to the severity of the fibrosis observed, the cases were classified as Group 0, no fibrosis; Group I, early fibrosis with slight collagen deposition, bile duct and capillary proliferation, and loss of hepatic parenchyma; Group II, intermediate fibrosis with focal collagenous scarring, and Group III, marked fibrosis with confluent collagenous scars and walling-off of nodules of the parenchyma.

Seventeen leukemic autopsies were studied during 1940-47, prior to the advent of the antimetabolites and adrenal steroids: 12 patients were in Group 0 and five in Group I. During 1948-51, when only amethopterin and the adrenal steroids were available, of 75 autopsies, 12 were in Group 0, 40 in Group I, 22 in Group II, and 1 in Group III. From 1952-1957, 6-mercaptopurine and azaserine were

used in addition to the adrenal steroids and amethopterin. Of 172 autopsies, 36 were in Group 0, 73 in Group I, 49 in Group II, and 14 in Group III. Of the 38 children with malignant neoplasms who received some form of chemotherapy including antimetabolites, 27 were in Group 0, 11 in Group II. The consistent increase in the incidence of hepatic fibrosis seems to correlate with the use of chemotherapeutic agents and prolonged survival time of leukemic patients.

These hepatic lesions will be compared with those occurring with hepatotoxins and with nutritional disturbances, such as Kwashiorkor.

174. The Effect of Immediate Versus Delayed Clamping of the Umbilical Cord on the Venous Pressure of the Newborn Infant

PAUL M. TAYLOR*; EDNA L. BIRCHARD*; NANCY H. BRIGHT,* and THOMAS J. EGAN,*
Pittsburgh and Elizabeth Steel Magee Hospital, Pittsburgh

Introduced by ROBERT KLEIN.

The finding of a significant but temporary elevation of umbilical vein pressure in a group of newborn infants at birth was reported by us at the meeting of this society two years ago. It was postulated then that this elevation of venous pressure might be due to overloading of the circulation by infusion of blood from the placenta associated with delayed ligation of the umbilical cord.

Preliminary to testing this hypothesis, an approximation of the volume of the "placental transfusion" was made. A mean difference of 70 ml. was found between the volume of blood obtainable from the placental fetal vascular bed in 15 term infants whose cords were clamped at delivery and in 15 term infants whose cords were clamped three minutes after birth. All infants in the latter group were held below the level of the perineum from birth to the time of clamping of the cord.

Umbilical vein pressures were then recorded on seven full term infants whose cords were clamped at the time of delivery and on eight full term infants whose cords were clamped at three minutes after delivery and who were held below the level of the perineum from birth. Venous pressure exceeded 120 mm. of saline in six of the eight late-clamped infants but did not exceed 120 mm. of saline in any of the early-clamped infants. The greatest difference between the mean values of the two groups was noted at six to nine minutes of age, when the late-clamped infants' value was 134 mm. of saline and the early-clamped infants' value was 86 mm. of saline.

The possible implications of these findings are being tested in a controlled clinical study.

* By Invitation

175. Evaluation of Factors Affecting the Intensity of Neonatal Jaundice in Premature Infants

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Pittsburgh and The Elizabeth Steel Magee Hospital, Pittsburgh

Introduced by ROBERT KLEIN.

It has been amply documented that the premature infant is more likely to develop intense neonatal jaundice than the term infant. The explanation of the marked variation in severity of jaundice among premature infants is poorly understood. This study was undertaken to attempt to delineate some of the factors influencing the intensity of neonatal hyperbilirubinemia in premature infants.

Eighty-one Negro and forty-seven white infants delivered at the Magee Hospital and weighing between 920 and 2500 gm. were studied. The umbilical cord was clamped at the time of delivery in half of the infants and after a three minute delay (during which time the infant was held below the level of the perineum) in the remaining infants. Respiratory rates for the first 48 hours, weight loss on the fourth day, maternal and perinatal factors were recorded. The concentration of serum bilirubin was determined between 48 and 72 hours in all infants. A-B-O or Rh immunization was ruled out in all cases.

The time of clamping of the umbilical cord did not influence the third day bilirubin levels, although venous hematocrits were significantly higher on the third day of life in the late-clamped group (mean=53.8±5.2) than in the early-clamped group (mean=45.9±6.6; $P<0.01$).

Mean bilirubin values varied inversely with birth weight and gestational age in both Negro and white infants. Bilirubin levels were higher in all weight groups for white than for Negro infants, but these differences were not significant. Only when compared on the basis of gestation of 36 weeks or less were significantly higher differences in white than Negro infants found: the <32 week group, $P=<0.01$, the 33 to 36 week group, $P=0.01$. The 37 to 40 week groups showed no significant difference. The possibility that the Negro infants might reach their peak bilirubin values more slowly than the white infants was excluded by performing daily bilirubin tests in infants of both races in the 33 to 36 week gestation group. It was found that the white infants attained their peak bilirubin values later than the Negro infants, and that the difference between the mean levels in the two races was greater on the fourth than on the third day of life.

Within each group of infants (as defined by race and length of gestation) a direct correlation between bilirubin levels and weight loss in excess of 10% of birth weight existed. As more white

infants (39%) than Negro infants (21%) had excessive weight loss in the combined <32 week and 33 to 36 week gestation groups, this may partially explain the observed differences between the bilirubin values in these groups.

Diabetes mellitus is the only maternal complication that we have found to be associated with a significant increase in neonatal jaundice in prematures. We found no correlation between elevated respiratory rates and the severity of neonatal jaundice.

176. The Localization and Measure of Phosphorus-32 in Nucleoside Polyphosphates

K. K. Tsuboi,* Cornell
Introduced by May G. Wilson.

Renewed interest in nucleotide research has been generated as a consequence of advanced techniques leading to the discovery of many new compounds within this group. Study of the metabolic function of these compounds may be facilitated in many instances by application of isotope techniques.

Radiochemical investigation of this group of compounds has been undertaken using the isotope, phosphorus-32. Procedures appropriate to the resolution of complex mixtures of tissue nucleotides in microquantities and in high radiochemical purity have previously been presented. Localization and measure of the isotope within nucleotides possessing more than one phosphate group (nucleoside polyphosphates and derivatives) required special consideration.

This report concerns appropriate procedures which have been recently developed allowing for a step-wise degradation of all known nucleoside polyphosphates, yielding products containing one less phosphate group, as shown:

$$\text{Nucleoside} \xrightarrow{(-PO_4)} \text{Nucleoside Triphosphate}$$

$$\text{Diphosphate} \xrightarrow{(-PO_4)} \text{Nucleoside Monophosphate.}$$

The degradation is accomplished directly on paper chromatograms and the products are subsequently resolved in a radiochemically pure state by development with appropriate solvents. From specific activity measurements on the isolated products, the relative incorporation of isotope and consequently the "turn-over" of each of the phosphate groups of the original nucleoside polyphosphate can be readily determined.

177. Placental Transport of Labeled Hydrocortisone to Infants with Congenital Adrenal Hypoplasia

Robert A. Ulstrom and Jane Burley,*
Minnesota

The prepartum diagnosis of fetal anencephaly was made in two pregnant mothers. Each infant was born alive. The first survived four hours; the second survived 40 minutes. Autopsies revealed, in addition to the anencephaly, that only small remnants of the adrenal glands were present in one infant and in the second infant no adrenal gland remnants could be found.

Each mother was given 3μc. of hydrocortisone-4-C14 intravenously within one hour preceeding vaginal delivery. Blood specimens were obtained from the mother's vein and the cord artery and vein simultaneously at parturition. Additional blood specimens were obtained from maternal and infant venous blood following parturition. The plasma was extracted with methylene chloride, and the extracted radioactivity was measured in a liquid scintillation counter. This radioactivity is representative of the circulating hydrocortisone and its metabolities present in the unconjugated form in the plasma. The ratios of maternal vein to cord vein radioactivity in this extract were 10:4 and 20:4 respectively. The ratios of radioactivity in the cord vein extract to the respective cord artery extract were 40:26 and 40:27.

Fractionation of the extracts was performed using florisil columns in one mother-infant combination. No radioactivity was eluted using chloroform. Using 2% methanol and 98% chloroform, 4% of the radioactivity from the maternal extract and 27% from cord vein extract was eluted, indicating that a much higher fraction of the steroid in the cord vein was composed of less polar unconjugated metabolites of hydrocortisone. The remainder was eluted by 25% methanol in chloroform. Paper chromatographic separation of extracts of infant's venous blood revealed the presence of material which traveled at an identical rate with hydrocortisone in one infant. When eluted from paper, this material was shown to possess the properties of radioactivity and fluorescence in sulfuric acid methanol. No such material was found in the second infant.

Further studies of chromatographically separated steroids indicate that only a small amount of the maternal hydrocortisone was transferred to these infants as specific hydrocortisone, and that the placenta either preferentially transports metabolites of hydrocortisone or the placenta itself metabolizes hydrocortisone during transport to the fetus. A relatively slow transport in the fetal-maternal direction would also explain this. The high percentage of less polar steroids in the cord vein plasma extract, together with the virtual absence of adrenal tissue in these infants, suggests that the placenta is the source of the high plasma value of 17-ketosteroids in an anencephalic infant with hypoplastic adrenals, reported by others.

* By Invitation

178. Electrocardiographic Changes in Newborn Premature Infants During Respiratory Distress Syndrome

ROBERT USHER,* McGill
Introduced by F. C. FRASER.

Serial electrocardiograms have been taken on fourteen newborn premature infants during marked respiratory distress. Between 12 and 48 hours of age, changes appeared in each case which were not present in tracings taken on 25 normal newborn prematures. These abnormalities increased in severity until death, or disappeared by age 36-to-72-hours in survivors.

The abnormalities included; 1. Prolongation of the PR interval beyond 0.11 seconds, with 2 to 1 atrioventricular block in two cases; 2. QRS duration of over 0.04 seconds; 3. Widening of the P wave; 4. Diminished QRS voltage throughout, most marked in standard Lead I; 5. Elevation of T waves in the standard leads and deep inversion in chest leads V_{1-3}; 6. Depressed ST segments in the chest leads V_{1-4}, and 7. Progression to bizarre complexes almost sine wave in nature in three cases, one of which lived.

Similar changes have been described with hyperkalemia in anuric patients, often preceding death due to cardiac effects of the metabolic disturbance. These abnormalities are known to be exacerbated by hyponatremia, and by metabolic acidosis. Blood chemical studies currently in progress seem to demonstrate metabolic acidosis, uremia, hyperkalemia, hyponatremia, and excessive weight loss in premature infants during respiratory distress. These metabolic changes are not associated with decreased urinary output of nitrogen or electrolytes and would seem to be the result of severe tissue breakdown. The above electrocardiographic findings may therefore be metabolic in origin, and they may indicate cardiotoxicity due to hyperkalemia and acidosis.

179. Electron Microscopy of Experimental Renal Disease

ROBERT L. VERNIER*; BENJAMIN PAPERMASTER*; ROGER ARHELGER*; FRED MECKLENBURG,* and ROBERT A. GOOD,
Variety Club Heart Hospital and
University of Minnesota.

Nephrosis is characterized by a consistent abnormality of the basement membrane revealed by electron microscopy. The abnormality observed consists of smearing of the epithelial foot processes along the basement membrane. This characteristic ultramicroscopic anomaly has been observed in all forms of nephrosis thus far studied in man.

In order to study the pathogenesis of the nephrotic syndrome, recourse has often been made to experimental models in which a nephrosis-like disease has been produced in several different ways. The models currently under study in our laboratory include:

1. Rat nephrotoxic serum disease in which an antirat-kidney serum, produced in the rabbit, is injected intravenously into rats;
2. Rabbit nephrotoxic serum disease in which an antirabbit-kidney nephrotoxic serum, produced in the duck, is injected intravenously into rabbits, and
3. Aminonucleoside nephrosis produced by daily subcutaneous injections of 6-dimethylamino-purine-3-amino-d-ribose (aminonucleoside).

It is the purpose of this report to compare lesions observed in each experimental model with the pathology observed in human diseases as revealed by both light and electron microscopy.

Renal disease developing in rats given aminonucleoside is associated with nephrotic syndrome featured by severe proteinuria beginning six days after injections are started. By light microscopy, the glomerulus shows normal morphology. Electron microscopic study reveals a consistent "smearing" of epithelial-cell foot processes along the glomerular basement membranes, strikingly similar to the ultramicroscopic change observed in various forms of nephrotic syndrome in man. The change observed begins about the seventh day after the injections are started and becomes progressively more severe. In the rat nephrotoxic serum disease, proteinuria begins soon after injection of the nephrotoxic serum in large doses. This syndrome is associated with light microscopic changes described as a proliferative glomerulonephritis. Electron microscopy of the kidneys from animals with this disease reveals proliferation of glomerular endothelium and accumulation of large amounts of basement membrane-like material within and between the endothelial cells. The epithelial cells and their foot processes remain intact early in the disease. When clinical findings of nephrotic syndrome are fully developed, the glomeruli from these animals show patchy changes in epithelial foot processes similar to those observed in some children with nephrotic syndrome associated with clinical and pathological chronic glomerulonephritis.

Rabbit nephrotoxic renal disease, produced with duck nephrotoxic serum, is characterized by proteinuria developing after a latent period of six to eight days. The clinical and morphologic findings are similar to those described in rabbit-antirat experimental glomerulonephritis. Electron microscopy reveals endothelial proliferation and basement membrane changes similar to those seen in rat nephrotoxic serum disease.

Electron microscopy of the early renal glomerular lesion of these three forms of experimental renal disease support the view that aminonucleoside

* By Invitation

nephrosis in rats is like human nephrosis in its ultramicroscopic pathology, while rabbit-antirat nephrotoxic serum disease and duck-antirabbit nephrotoxic serum disease are more comparable to glomerulonephritis in their ultramicroscopic pathology.

Preliminary studies of proteinuria induced in dogs by extreme elevation of serum protein levels produced by constant intravenous infusion of dog plasma show that "threshold" proteinuria does not result in significant morphological changes in the glomerulus by either light or electron microscopy.

180. A Preliminary Report on the Use of a New Oral Hypoglycemic Agent in Juvenile Diabetes

W. B. Weil Jr., Western Reserve

N'-β-Phenethylformadinyliminourea hydrochloride (DBI) given orally has been shown to exhibit hypoglycemic activity in diabetes at all ages. Substitution of DBI for insulin has been evaluated in a small group of children 6 to 13 years of age with a duration of diabetes from six months to four years and taking 20 to 70 units of insulin daily. Complete replacement of insulin could be obtained using 4 to 8 mg. of DBI per unit of insulin. Increasing dosage of DBI led to the progressive gastrointestinal symptoms of anorexia, abdominal pain, and vomiting with early appearance of ketonuria. These effects appeared at dosage levels of 50 to 150 mg. per day. Positive cephalin flocculation tests occurred in several children without other evidence of abnormal liver function. None of these patients were able to be maintained for more than a day or two on the oral compound alone because of the above-mentioned symptoms.

181. Failure to Induce Dietary Deficiency of Copper in Premature Infants

J. Wilson * and M. E. Lahey, Cincinnati

A type of anemia occurring in infants and showing impressive morphologic and biochemical similarities to the anemia of copper-deficient animals has recently been recognized. Since the affected subjects had subsisted largely on milk, and since current methods of processing milk maintain the copper content at a low level, the possibility that a dietary deficiency of copper might occur in the human subject has again been raised.

In an attempt to evaluate the role of the diet in the genesis of this type of anemia, a feeding experiment was undertaken. Premature subjects weighing less than 3 lb. at birth were purposely chosen because their "copper stores" are scanty and because they grow at rates approaching those of certain experimental animals. Both of these factors might be expected to make the prematurely born infant especially vulnerable to a reduction in the copper content of the diet.

All subjects were fed nothing but milk and vitamin supplements for 9 to 14 weeks. One group of seven infants received less than 20μg. of copper per kilogram body weight per day. Another group of five infants received from 5 to 50 times this amount. The results indicate that the levels of copper and iron in the serum and of hemoglobin in whole blood were essentially the same in the two groups. The characteristic triad of copper deficiency in animals, i. e., microcytic hypochromic anemia, hypocupremia, and hypoferremia, was not encountered during the period of milk feedings and did not appear in subsequent months. It was thus not possible to produce a dietary deficiency of copper in premature infants under the conditions of the experiment.

* By Invitation

182. Radioiron Uptake in Iron Deficiency

Calvin W. Woodruff, Vanderbilt

The utilization of therapeutic doses of ferrous sulfate for hemoglobin formation has been studied in a group of 25 patients with hypochromic anemia ranging from 6 to 18 months in age. These patients formed a homogeneous group, all having a hemoglobin concentration of less than 9.0 gm. per 100 ml., hypochromia, microcytosis, low serum iron concentrations and high total iron-binding capacity. Fe^{59} of high specific activity was added to enough ferrous sulfate solution (Fer-in-Sol) to permit the administration of amounts of iron varying from 0.25 to 4.0 mg. per kilogram containing approximately 0.1μc. per kilogram of radioactivity. The labeled ferrous sulfate was administered in 2 oz. of orange juice following a four hour fast. Blood was obtained 10 to 14 days later for the determination of radioactivity. The total activity was calculated from assumed blood volumes, and the utilization of iron for hemoglobin formation was expressed as per cent of the administered dose. An inverse relationship between the amount of iron given and per cent uptake was found. For doses of 0.25 to 0.50, .75 to 1.5, 2.0, and 4.0 mg. per kilogram the uptake was 24.6%, 19.6%, 13.4%, and 9.1% respectively. The net uptake of iron for these same groups was .107, .207, .269, and .379 mg. per kilogram.

The percent uptake of iron in these patients appears to bear a consistent relationship to the logarithm of the dose of iron expressed as milligrams per kilogram. At the higher dosage levels studied, the net uptake of iron tends to level off with increasing dosage, and the most efficient single dose appears to be less than 4.0 mg. per kilogram. The implications of these observations in the treatment of iron deficiency will be discussed.

183. The Treatment of Glycogen Storage Disease with Glucagon

R. YIM *; J. MACLAUGHLIN,* and S. P. BESSMAN, Maryland

Since glucagon stimulates the enzymatic breakdown of glycogen, a rational method of therapy of the excessive storage of glycogen in hepatic glycogenosis suggests itself. Although short term use of glucagon by other investigators for increasing blood sugar has not been successful, it might be possible to improve liver function by accelerating glycogenolysis by this hormone.

Studies of blood lactic acid during galactose and glucagon tolerance curves serve to reveal the function of the glycolytic pathway and permit a degree of prediction as to the possible efficacy of glucagon therapy.

The glucagon is given in order to stimulate the conversion of glycogen to lactic acid and possibly to impede the synthesis of glycogen. The lactic acid excess is excreted in the urine.

The use of glucagon for almost one year in a case of glucose-6-phosphase deficiency and for shorter periods in cases of the abnormal type glycogen and cardiac glycogenosis will be described. Clinical improvement has been seen only in the first type with glucagon therapy.

Differential techniques for clinical identification of types of glycogenosis will be discussed.

* By Invitation

News and Comment

ANNOUNCEMENTS

Annual Institutes on Maternal and Child Health and Crippled Children.—The Schools of Public Health of the Universities of Michigan and Minnesota announce that the first of a series of Annual Institutes on major problems in Maternal and Child Health and Crippled Children will be held in Minneapolis on Dec. 8-12, 1958. The topic for this first Institute will be "Perinatal Casualties—A Challenge to Maternal and Child Health and Crippled Children's Services." The Institute is also sponsored by the Michigan and Minnesota State Maternal and Child Health and Crippled Children's Services and has been made possible by a grant from the U. S. Children's Bureau.

The Institute will be held primarily for key health personnel on a multidisciplined basis working in State Maternal and Child Health and Crippled Children's Services in Regions V (Illinois, Indiana, Michigan, Ohio, Wisconsin) and VI (Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, South Dakota). All travel and living expenses and tuition fees will be paid by the University of Minnesota from funds granted by the Children's Bureau. Personnel attending the Institute will be selected by the State Directors of Maternal and Child Health and Crippled Children.

For further information, personnel in Region V may write to Dr. Donald Smith, Associate Professor of Maternal and Child Health, University of Michigan School of Public Health, Ann Arbor, Mich.; personnel in Region VI may write to Dr. Helen M. Wallace, Professor of Maternal and Child Health, University of Minnesota School of Public Health, Minneapolis.

Staphylococcal Infections.—The National Library of Medicine has compiled a lengthy bibliography on staphylococcal infection, which, it is hoped, may be of value to both private and public health physicians who are engaged in combating the increased incidence of antibiotic resistant staphylococcal infection in the home, community, and hospital. The bibliography will be sent at no cost on request to National Library of Medicine, 7th St. and Independence Ave., S. W., Washington 25, D. C.

Wyeth Pediatric Fellowships Awarded to Twenty Physicians.—The names of 20 recipients of the annual Wyeth Laboratories pediatric residency fellowships were announced today by Dr. Philip S. Barba, past president of the American Academy of Pediatrics and chairman of the fellowship selection committee.

The physician-recipients, from thirteen different states, are the first group to benefit from the fellowship program established recently by the Philadelphia pharmaceutical firm. Each doctor will receive a grant of $4800 providing for a two-year postgraduate course in pediatrics. The cost of the first three years of the project will be $195,000.

Those receiving the fellowship awards included Drs. S. Wayne Klein, Baltimore; Robert B. Cole, Cleveland; Floy C. Helwig, Baltimore; Warren D. Grover, Camp LeJeune, N. C.; Luther B. Travis, Fort Benning, Ga.; John D. Siegfried, Red Lake, Minn.; George T. Kimbrough, Birmingham, Ala.; Donald E. Carey, New Orleans; Marvin L. Rallison, Minneapolis; Allan L. Drash, Baltimore; William G. Kenney, Rochester, N. Y.; Paula Neyman, New York; Keith Hammond, Paoli, Ind.; Robert O. Hickman, Salt Lake City; John C. Gilliland, Jackson, Miss.; Jon L. Karlsson, Santa Rosa, Calif.; John I. Larkin, Albany, N. Y.; William A. Moon Jr., Denver; George A. Lentz, Baltimore, and Paul J. Uhlig, Oceanside, Calif.

Selection was made on the basis of interest in pediatrics, character, conscientious and able performance of duties, and academic competence.

Those receiving the pediatric fellowships may attend any hospital whose residency training program is properly accredited.

In announcing the recipients, Dr. Barba cited the Wyeth program for providing young physicians with the opportunity to obtain advanced education that will enable them to minister to both the physical and emotional needs of children, utilizing the most modern methods and techniques.

Other members of the selection committee include Drs. John A. Anderson, Minneapolis; Amos Christie, Nashville, Tenn.; Hugh A. Carithers, Jacksonville, Fla., and Dr. Crawford Bost, San Francisco.

Medical Research Program.—To help support the search for new medical knowledge and to increase the number of trained medical researchers, the National Fund for Medical Education has established a Medical Research Program through which contributions from united funds, community chests, voluntary health agencies, and other organizations will be channeled into basic research, Fund President S. Sloan Colt announced yesterday.

The United Community Funds and Councils of America, whose local affiliates have expressed a desire to help support broad fundamental programs of basic medical research on a national level, have agreed to support this new research program.

It is our hope, Mr. Colt said, that the voluntary health agencies which have so generously supported the categorical research into specific diseases will also support this new program.

This expansion of the National Fund program, Mr. Colt stated, will in no way affect its campaign among corporations to obtain support for the teaching budgets of the accredited medical schools in the United States.

Mr. Colt, who recently retired as chairman, Bankers Trust Company, New York, declared that the contributions sought for the new program are over and above local contributions for research in specific diseases: cancer, heart, polio, cerebral palsy, and others. He continued:

"By supplementing categorical research into the specific diseases this research program of the Fund will help to broaden overall research activities, and will be an important stimulus to the advancement of the medical sciences.

"The new program, it is expected, will not only help advance the search for basic medical knowledge and encourage the training of research personnel but will also help the nation's medical schools and other qualified institutions maintain a proper balance between basic and project, or applied, research."

The greatest need in research today, Mr. Colt continued, is for the support of basic research and the training of research personnel. The bulk of research money available at present is for project research. Creative research—the exploratory probing and groping in the unknown—too often must be financed out of meager general funds.

In 1952, Mr. Colt pointed out, a committee of medical educators published the results of a five-year study of medical education in which they declared that, in the financing of medical research, the most urgent need is for institutional—not project—research.

Because a major portion of medical research is carried on in the medical schools, whose faculties both teach and conduct research, Mr. Colt said he was confident that the new program would help many schools to improve further the quality of undergraduate medical education. Fund grants, at present principally supported by corporation contributions, are unrestricted but are used primarily for support of teaching budgets of the medical schools. Today, pure research and applied research supplement each other and both are important elements in the educational function of the schools.

Details of the new program were worked out by a committee composed of the following leading businessmen and educators: Dr. Vernon W. Lippard, Dean, Yale University School of Medicine, Chairman; S. Sloan Colt, President, National Fund for Medical Education; Dr. James M. Faulkner, Medical Director, Massachusetts Institute of Technology; Dr. Joseph C. Hinsey, Director, New York Hospital-Cornell Medical Center; Mr. T. V. Houser, Former Chairman, Sears Roebuck & Co.; Dr. Carlyle Jacobsen, President, State University of New York College of Medicine at Syracuse; Devereux C. Josephs, Chairman, New York Life Insurance Company; Dr. John McK. Mitchell, Dean, University of Pennsylvania School of Medicine; Eustace Seligman, Partner, Sullivan & Cromwell; Juan T. Trippe, President, Pan American World Airways; Dr. W. Clarke Wescoe, Dean, University of Kansas School of Medicine.

The National Fund for Medical Education was formed in 1949 under the leadership of President Dwight D. Eisenhower, then president of Columbia University; former President Hoover, who is honorary chairman of the Fund's Board of Trustees; leading educators, university presidents, and prominent business leaders.

The Fund recently awarded $3,178,825 to the medical schools, bringing to $15,843,766 the total awarded since its first grants were made, in 1949.

Books

BOOK REVIEWS

Pediatric Gynecology. By Goodrich C. Schauffler, M.D. Fourth edition. Price, $9. Pp. 349, with 84 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1958.

The standard reference dealing with gynecologic problems in children and young adolescents is "Pediatric Gynecology" by Goodrich C. Schauffler. The new edition reflects the author's endeavor to keep the book as current as possible and to incorporate the substantial increase of knowledge in this field. Dr. Schauffler's long-standing interest in the gynecologic problems of the immature and maturing female has resulted in his accumulation of a substantial clinical experience. He writes, therefore, with considerable authority on certain facets of his subject. Students and practitioners in both specialties can gain much from careful study of the descriptions of the anatomy and physiology of the genital tract and the acceptable deviations from the normal. They should readily develop a satisfactory technique of examination based on the author's careful and considerate procedure.

When allied specialties are discussed, co-authors have been wisely and freely used. However, one quickly arrives at the conclusion that the sections dealing with the manifestations of emotional problems in childhood do not reflect the views of competent child psychiatrists and that the addition of a co-author in this specialty would add balance that is clearly lacking. There is no mention of the various stages of development of the personality and their relation to the normal and abnormal function of the genital tract. The potentialities of psychoanalytic psychiatry are ignored in favor of the suggestion that a psychologist can frequently be of help in management of emotional disturbances.

Similar clarification might be sought in the discussion of pseudohermaphroditism and intersexuality, admittedly a subject which does not long remain static.

The discussion of vaginitis has benefited from decreased emphasis on gonorrhea and recognition that other types of vaginitis are more likely to be encountered in the average practice than that due to Neisseria. The discussion of the technical aspect of preparation of smears and cultures seems irrelevant to the needs of most physicians.

The publishers have prepared a volume that is easily readable, virtually free of errors, and of good quality. Illustrations are generally pertinent and are well reproduced.

The book can be recommended for its delineation of normal and abnormal anatomy and physiology, but the reader might profitably supplement the text with appropriate contemporary literature concerning child psychiatry and developmental abnormalities.

ELIZABETH MUSSEY, M.D.

The Nursing Mother. By Frank Howard Richardson. Price, not given. Tupper and Love, 55 5th Ave., New York 3, 1958.

As Dr. Richardson emphasizes, infants should be breast-fed up to the age of six months if possible, because human milk is the best milk for young infants. It has preventive and therapeutic value and is economical, easily digestible, fresh, free from contamination, automatically produced, and time-saving. It is the safest food for prematures who are mature enough to suckle at the breast. Instances of breast milk "not agreeing with an infant" are almost unknown. Breast feeding provides gratification and a sense of security to babies. Breast feeding also is important physiologically and psychologically to mothers.

The only contraindications to breast feeding are heart, kidney, lung, and psychiatric diseases in the mother. Also, she should not take drugs which pass into her milk, e. g., antibiotics, atrophine, barbiturates, bromides, iodides, morphine, salicylates, and sulfonamides.

Most mothers can nurse their infants if they have the desire, proper pre- and postpartal care, adequate sleep, moderate exercise, freedom from worry, and a good pre- and postpartal diet. They should drink one quart of milk and an additional quart of fluid daily and eat fruit, salads, green vegetables, eggs, meat, cod liver oil or its equivalent, and whole wheat bread in three to four well-balanced meals and rest at least one hour daily in addition to eight hours at night.

Normal infants suckle and swallow at birth; inability to nurse well is more likely to be due to an imperfect central nervous system than to muscular weakness, except in prematures.

This book is highly recommended to general practitioners, obstetricians and pediatricians.

W. C. DAVISON

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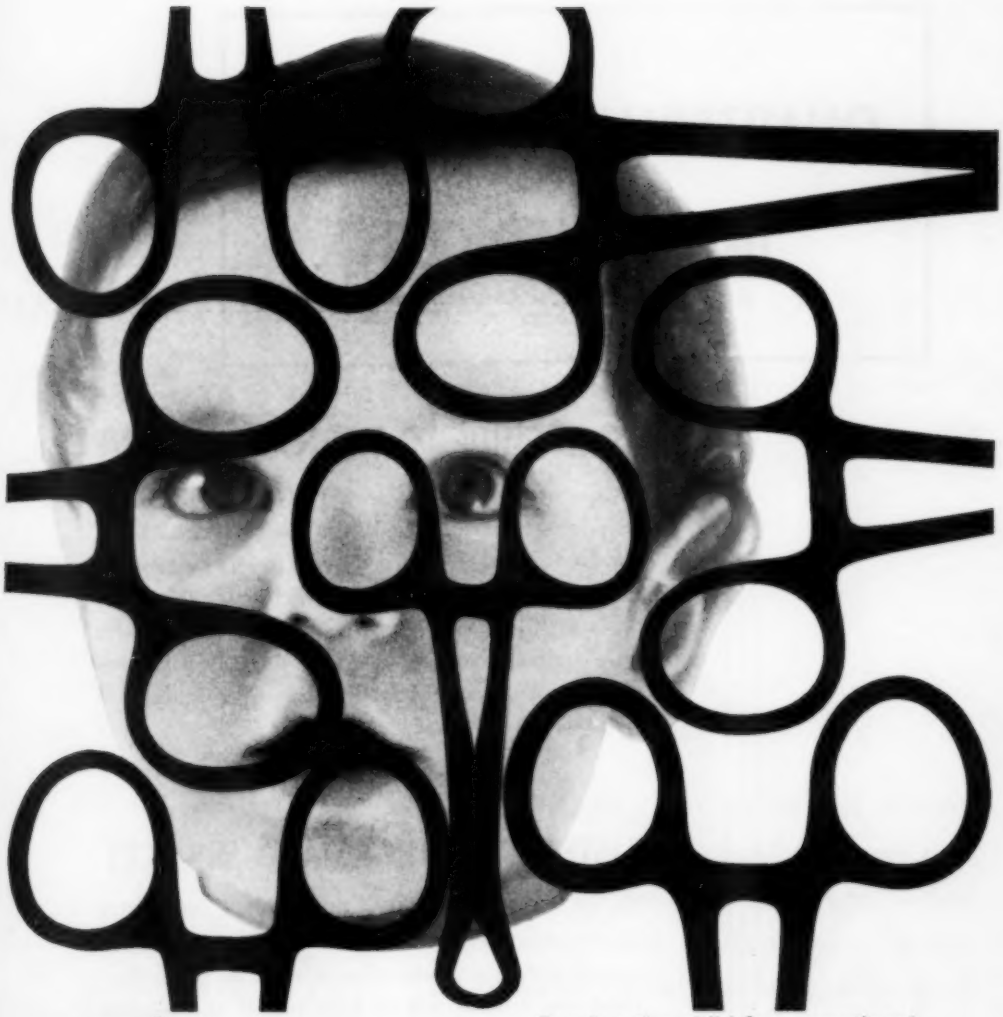

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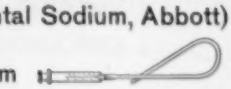

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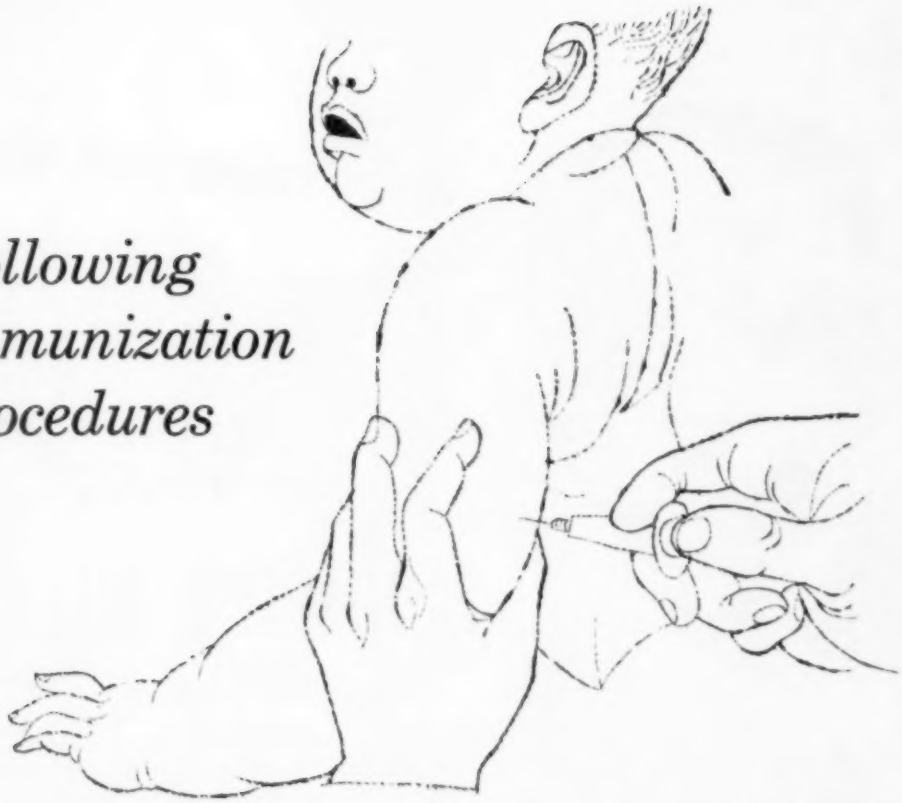

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